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Genetic and demographic signatures of population fragmentation in a cooperatively breeding bird from south-east Kenya.

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Front cover photograph:

Cabanis's greenbul (*Phyllastrephus cabanisi*) on a nest in the Taita Hills © Alexander Callens

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Martine Van Tongel

Genetic and demographic signatures of population fragmentation in a cooperatively breeding bird from south-east Kenya.

Genetische en demografische effecten van populatie versnippering in een coöperatief broedende vogelsoort van Zuidoost-Kenia.

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Sunrise in the Taita Hills. © Tom Callens

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Intact indigenous cloud forest interior in the Taita Hills (Mbololo forest). © Tom Callens

Habitat loss and fragmentation constitute the primary causes of worldwide extinctions and biodiversity loss (Turner 1996, Laurance and Bierregaard 1997, Pimm and Raven 2000). Based on estimations of The Food and Agriculture Organization of the United Nations, the world has lost approximately 40% of its originally 60 million km² of forest cover (FAO 1997). Although global deforestation rates have been decreasing during the last decade from 16 million hectares per year (1990-2000) to 13 million hectares per year (2000-2010), the process still continues at an alarming rate (FAO 2011). Habitat loss and fragmentation typically result in smaller and more isolated populations of plants and animals, which are further suppressed by stochastic genetic, demographic and environmental processes (Frankham *et al.* 2005). The combination of these stochastic factors can synergistically lead to extinction, the so-called extinction vortex (*sensu* Gilpin and Soulé 1986).

Recurrent mating among relatives in small and isolated populations can lead to heterozygosity loss and the accumulation of mildly deleterious alleles (Frankham 1995, Higgins and Lynch 2001, Frankham *et al.* 2002), possibly resulting in reduced reproduction and survival (Keller and Waller 2002, Frankham 2005). Apart from inbreeding (depression), genetic variation may be lost through random genetic drift, whereby some alleles will become fixed and others will be lost, at a rate inversely related to population size. Rates of genetic stochastic processes are not determined by the actual number of individuals alive (census population size, N_c), but rather by the population's effective size (N_e) (Crow and Kimura 1970, Kalinowski and Waples 2002, Palstra and Ruzzante 2008). This effective size (N_e) reflects the size of an 'ideal' population that would have the same rate of genetic change as the population under consideration, assuming equal sex-ratio, equal variance in reproductive success, random mating, etc. (Wright 1931). Because natural populations rarely comply with these 'ideal' assumptions (Whitlock and McCauley 1999), the effective population size is usually less than the census size (Caballero 1994, Leberg 2005). N_e hence constitutes a key parameter in the genetic study of small populations, since low values imply that such populations become more sensitive to genetic stochasticity at levels where census population sizes would not

provide such indications (Palstra and Ruzzante 2008). Apart from short-term effects, loss of genetic variation may also adversely affect population viability on the longer term by reducing the evolutionary potential of species or populations, i.e. their ability to adapt to environmental change or geographically varying local conditions (Madsen *et al.* 1996, Reed and Frankham 2003, Frankham 2005), such as evident under global environment change.

A crucial factor for counterbalancing genetic and demographic stochastic effects in small-sized populations is dispersal, as immigrants can counteract genetic stochastic processes by increased genetic variation through gene flow ('genetic rescue', *sensu* Ingvarsson (2001)), and demographic stochasticity by compensating for low survival and/or birth rates of residents, thereby decreasing the probability of local extinction ('rescue-effect', *sensu* Brown and Kodric-Brown 1977, Hanski and Gilpin 1991). Though contemporary dispersal can be estimated through mark-recapture studies, such studies are often labour-intensive and expensive, based on small sample sizes, and plagued by the uncertainty of whether or not dispersal is genetically effective (Koenig *et al.* 1996). Genetic studies, in contrast, are more powerful in assessing dispersal and gene flow, either directly by measuring dispersal over one or a few generations by genetic assignment tests (Manel *et al.* 2003, Mank and Avise 2004), or indirectly by inferring genetically effective dispersal (gene flow) over several to thousands of generations with F_{ST} or D_{est} statistics (Allendorf and Luikart 2007, Broquet and Petit 2009). Quantifying spatiotemporal variation in effective population sizes, genetic diversity and dispersal or gene flow is therefore essential to uncover the underlying demographic and genetic processes in fragmented populations and to design conservation plans that may help to prevent population declines and/or extinction.

While stochastic genetic processes are important drivers of population viability through the effects of inbreeding depression and loss of genetic variation, these threats are only a part of the challenges faced by species in the wild. For example, human influence on natural landscapes that results in reduced fragment sizes, increased fragment isolation, increased edge-to-interior ratio of remnants, and alterations of the remnants' habitat structure, may directly or indirectly contribute to changes in nest predation rates on eggs and nestlings (Flaspohler *et al.* 2001, Tewksbury *et al.* 2006). This, in turn, can affect the reproductive success of nesting birds (Wilcove 1985, Martin and Roper 1988, Sieving 1992), sometimes resulting in negative population growth rates and potentially reducing their long-term viability if such trends persist for a long time (Robinson *et al.* 1995, Flaspohler *et al.* 2001). Predation

can be affected at multiple spatial and temporal scales (Stephens *et al.* 2003, Thompson 2007). At landscape scale, high nest predation rates are often associated with increased fragmentation and anthropogenic disturbance (Small and Hunter 1988, Robinson *et al.* 1995, Tewksbury *et al.* 2006), while at habitat and nest-site scales, nest predation may vary with distance to the habitat edge (Gates and Gysel 1978, Andrén and Angelstam 1988, Lahti 2001, Batáry and Báldi 2004), vegetation cover, nest height and nest concealment (Martin 1992, 1993, Lambert and Kleindorfer 2006, Colombelli-Négrel and Kleindorfer 2009). At a temporal scale, factors affecting nest predation rates may include year, timing of breeding (Mezquida and Marone 2001, Peak 2007) and nest stage (egg laying, incubation, nestling) (Peak *et al.* 2004, Grant *et al.* 2005, Brawn *et al.* 2011) or nest age (Grant *et al.* 2005, Low and Pärt 2009). These temporal factors, in turn, are often associated with variation in abundance, diversity, prey selection or behavior of predators (Chalfoun *et al.* 2002, Estrada *et al.* 2002, Peak 2003, Kvarnbäck *et al.* 2008, Sperry *et al.* 2008), or different cues provided by prey (eggs or nestlings) or parents (Redondo and Castro 1992, Haff and Magrath 2011). Quantifying predation rates at different spatiotemporal scales is crucial when aiming to build predictive models of avian population dynamics under different scenarios of habitat change (Robinson *et al.* 1995, Chalfoun *et al.* 2002, Grant *et al.* 2005).

In addition to stochastic and deterministic populations threats, specific behavioral traits, such as cooperative breeding, might render a species unusually vulnerable to fragmentation, or adversely, more resistant to it (Walters *et al.* 2004). Cooperative breeding birds are species where the breeding pair is assisted by conspecifics helping to care for offspring that are not their own (Brown 1987, Cockburn 1998, Koenig and Dickinson 2004). The majority of cooperative breeders exhibit delayed dispersal of offspring, thereby foregoing their own reproduction and becoming non-reproductive helpers of parents or other close relatives (Ekman *et al.* 2004, Dickinson and Hatchwell 2004, Hatchwell 2009). These helpers do not only delay dispersal, but also show altered dispersal behavior by waiting to take over the breeding position on the natal territory or looking for possibilities in a nearby territory during short expeditions from the natal territory. This dispersal strategy is called 'stay-and-foray' (SAF) (*sensu* Brown 1987) in contrast to the more classic strategy whereby birds continuously depart to search for vacancies or 'depart-and-search' (DAS) (*sensu* Brown 1987). Cooperative breeding species generally exhibit a greater proportion of individuals employing SAF rather than DAS, which leads to an overall skew towards short dispersal distances (Walters *et al.* 2004). The differences in dispersal patterns between cooperative and non-cooperative breeder

forms the basis for differences in their sensititivity to habitat fragmentation. The level of vulnerability of cooperatively breeding species to habitat loss and fragmentation is function of several factors, i.e. the average fragment size in the landscape, the impact of the landscape matrix on inter-fragment dispersal (functional connectivity), the distances between fragments relative to helper dispersal distance and the fraction of individuals employing DAS (Walters *et al.* 2004). In large fragments cooperative breeders are usually more abundant than pair-forming species, for two reasons: first, the presence of large numbers of helpers is expected to increase the rate of (re)colonization of vacant breeding territories, and second, pools of replacement breeders are expected to dampen fluctuations in the size of breeding population and hence to counteract adverse population effects caused by demographic and environmental stochasticity (Walters *et al.* 2004). However, the situation may be opposite in small, isolated fragments that are beyond the dispersal range of other fragments. As cooperative breeders usually live in groups consisting of close relatives (Ekman *et al.* 2004, Dickinson and Hatchwell 2004), strong isolation may result in increased chances of incestuous mating, and hence, in higher risks of inbreeding depression (Koenig and Haydock 2004). Most species have been shown to dispose of mechanisms to avoid or reduce inbreeding, such as sex-biased dispersal, in which females generally leave their natal patch to avoid paring with a related male (Koenig and Haydock 2004, Komdeur 2004, Double *et al.* 2005, Temple *et al.* 2006, Hatchwell 2009). However, in such case, filling breeding vacancies will depend primarily on individuals performing between-fragment dispersal (DAS). Since only a small proportion of the population employs DAS, a much smaller number of individuals will be available to fill breeding vacancies than in non-cooperative species, which may result in restricted matings and reduced reproduction and population growth in small and isolated fragments (Walters *et al.* 2004). Cooperative breeding species can hence be predicted to run lower risk of extinction than pair-forming species in large, isolated habitats, but higher risks in small, isolated fragments (Walters *et al.* 2004).

In the tropical region, an estimated 50% of the total indigenous forest cover has been lost due to anthropogenic activities (Pimm and Raven 2000, Lewis 2006), with the highest rates of loss currently occurring at the African continent (FAO 2011). In contrast to the temperate region, anthropogenic disturbance of such a magnitude did not occur in the recent past, and the ensuing absence of past extinction filters (Hannah *et al.* 1995, Balmford 1996), in combination with high levels of ecological specialization (Henle *et al.* 2004, Brook *et al.* 2008), renders tropical forest species particularly vulnerable to contemporary human-induced

pressure. Although temperate and tropical species face comparable challenges following the loss and fragmentation of their habitats, mechanisms underlying observed decreases in bird diversity may differ, and hence, conservation strategies that have proved effective in the temperate regions, may be less effective in the tropics (Stratford and Robinson 2005). One problem in this respect is the general lack of detailed, long-term population studies in the tropics, which makes it difficult to measure and predict impact of habitat loss and fragmentation on tropical bird species (Sodhi *et al.* 2011). For example, predation rates are expected to be higher in fragmented tropical compared to temperate forests (Robinson *et al.* 2000, Stutchbury and Morton 2001), because this geographical region hosts a much more diverse suite of nest predators, including many species of reptiles, mammals and birds (Skutch 1985, Roper and Goldstein 1997, Robinson *et al.* 2005a). Despite the fact that tropical bird species are generally characterized by higher survival rates, longer lifespans, smaller clutch sizes and lower rates of fecundity compared to their temperate-zone counterparts (Johnston *et al.* 1997, Stutchbury and Morton 2001, Peach *et al.* 2001), evidence from population viability studies is growing that high levels of nest predation rates can severely decrease the life-time reproductive success of tropical bird species too, and ultimately result in unviable populations (e.g. Heinsohn *et al.* 2009). Yet, because detecting and monitoring large numbers of natural nests is often difficult in dense and richly-structured tropical forests (Sodhi 2009), studies of tropical nest predation are often inferred from artificial nests, despite the fact that the latter are believed to only poorly mimic natural ones (Moore and Robinson 2004, Robinson *et al.* 2005b).

Since the initiation of the Taita Hills Biodiversity project in 1996 (Bytebier 2001), several studies have been executed to understand effects of habitat loss, fragmentation and detoriation on Afrotropical bird species. The Taita Hills are particularly suitable to study bird populations in a context of forest fragmentation because the area is surrounded by over 80 km of unsuitable habitat, hence comprising a 'closed' (meta)population system. Ecological processes and patterns of persistence have primarily been derived from comparative multi-species studies (Lens *et al.* 1999, 2002b; Githiru *et al.* 2002, 2005b; Adriaensen *et al.* 2006; Lens 2007; Lehouck *et al.* 2009a,b), while more detailed studies mainly focussed on critically endangered species (e.g. *Turdus helleri*: Lens *et al.* 1998, Galbusera *et al.* 2000, Lens and Van Dongen 2002, Lens *et al.* 2002a; *Apalis fuscigularis*: Borghesio *et al.* 2010) or species that have not been adversely affected by forest fragmentation (e.g. *Zosterops (poliogastrus) silvanus*: Mulwa *et al.* 2007; *Pogonocichla stellata*: Galbusera *et al.* 2004; Githiru *et al.*

2005a, 2006, 2007a; Githiru and Lens 2006a,b; Spanhove *et al.* 2009a,b). Insights from studies on common species (e.g. *Pogonocichla stellata*) can be useful in understanding the population dynamics of more endangered species (e.g. *Turdus helleri*, *Apalis fuscigularis*), and hence our ability to indicate proper conservation actions (Githiru and Lens 2004, Githiru *et al.* 2007b). However - besides *P. stellata* - limited detailed information was available on other sympatric bird species that are highly forest-restricted and affected by fragmentation, but still sufficiently abundant to derive ecological insights and act as surrogates to guide conservation for ecologically similar species. Additionally, no accurate assessments of changes in genetic and demographic parameters over a contemporary timescale have been made, despite the potential effects of enhanced forest protection and awareness over the past decade (Githiru *et al.* 2011). Furthermore, even though there are preliminary indications of cooperative breeding in *P. cabanisi*, there has been little interest in species displaying such complex behavior, although they might be particularly sensitive to habitat fragmentation (Walters *et al.* 2004). Hence, there was an urgent need for a long-term in depth study evaluating genetic and demographic effects of population fragmentation on an intermediately sensitive, forest-dependent cooperatively breeding bird species over a recent ecological timescale.

OBJECTIVES AND OUTLINE

Starting from the evident lack of detailed studies on genetic and demographic effects of habitat fragmentation in tropical ecosystems, and on possible conservation implications stemming from the fact that tropical and temperate-zone species differ in life history traits, we conducted a four-year field study in south-east Kenya with the implicit aim of advancing our understanding on how tropical rainforest fragmentation may lead to population fragmentation in cooperatively breeding, forest-restricted bird species. To achieve this aim, we studied genetic and demographic signatures of rainforest fragmentation on isolated populations of the cabanis's greenbul (*Phyllastrephus cabanisi*), the results of which are described in five consecutive manuscripts. In **Chapter 1**, historical shifts in mobility in three *P. cabanisi* populations are evaluated against those of six sympatric forest bird species. This is achieved by comparing species-specific levels of past population differentiation, estimated from sets of microsatellite genotypes, with contemporary dispersal rates, estimated from multi-strata capture-recapture models. In **Chapter 2**, shifts in demographic and genetic properties within and between five *P. cabanisi* populations are quantified over a recent, fifteen year time interval and linked to current levels of population connectivity. Because forest fragmentation and disturbance may also affect population dynamics through changes in patterns of nest predation rates, **Chapter 3** describes spatial and temporal variation in predation rates on natural nests of *P. cabanisi* in two contrasting forest fragments during three consecutive breeding seasons. Based on detailed nest observations (video-analyses) during these nest surveys, in **Chapter 4**, the social breeding behavior of *P. cabanisi* is examined, together with detailed quantification of variation in food provisioning rates by helper birds at the nests, and possible fitness effects thereof for the adult breeders. Since close kin aggregations associated with cooperative breeding may result in fine-grained spatial genetic patterns that remain undetected by the use of traditional population-level tools (Chapters 1, 2), **Chapter 5** revisits the genetic signature of forest fragmentation by adopting an individual-based approach at different spatial scales. In a final chapter, results described in these five manuscripts are discussed and integrated within an ecological and conservation framework, and this discussion results in a number of recommendations for future tropical studies.

STUDY SPECIES

Cabanis's greenbul *(Phyllastrephus cabanisi*; Photo 2, 3A&B), member of the family of the *Pycnonotidae* (bulbuls) (Keith *et al.* 1992), is a medium-sized forest-dependent passerine that inhabits central- to east-African (sub)tropical lowland to montane moist forest (Keith *et al.* 1992; Bennun *et al.* 1996). Globally, the IUCN conservation status of the species is currently evaluated as 'Least Concern' (Birdlife International 2012). Noisy flocks usually forage in the understory and near *Dorylus* ant swarms and sometimes higher, including canopy of small trees (Keith *et al.* 1992). The species shows no sexual dimorphism, though males are usually larger in size (♂: wing length 78-92 mm; tarsus length 22-23 mm; ♀: wing length 74-85 mm; tarsus length 21-23 mm) (Keith *et al.* 1992). The birds are monogamous and mated pairs usually remain together during several seasons (Keith *et al.* 1992). The species lives in small family groups whereby fledged young stay with their parents during the post-fledgling period, even up to the next season (Keith *et al.* 1992). Besides insects, *P. cabanisi* occasionally feeds on fruits (Keith *et al.* 1992, Githiru *et al.* 2005b), but supplies nestlings with invertebrates (Keith *et al.* 1992) and even small vertebrates such as little tree frogs and lizards (T. Callens, *pers. obs.*). The open cup-shaped nests are constructed by both sexes, typically at 1-1.5m, but rarely higher than 5m above the ground (Keith *et al.* 1992). Nests are hidden with bits of bark and leaves hanging outside as camouflage, giving the impression of a mass of leaf debris caught in a fork (Photo 1A&B, Keith *et al.* 1992). In the Taita Hills, the female lays a clutch size of 2, exceptionally 3 eggs (22x15mm; Keith *et al.* 1992) which are incubated by both sexes but mainly ♀ for 17 days with most nestlings fledging after another 11-13 days (see Chapter 4). *P. cabanisi* individuals are generally very sedentary, occupying the same territory for several years (Keith *et al.* 1992). These birds have a long lifespan, with the oldest individuals reaching an age of at least 13 years (long term ringing data Taita Hills).

Photo 1. Cabanis's greenbul (*Phyllastrephus cabanisi*) nests in the Taita Hills. © Tom Callens

Photo 2. Cabanis's greenbul (*Phyllastrephus cabanisi*), captured in Mbololo (Taita Hills). © Tom Callens

Photo 3. Cabanis's greenbuls (*Phyllastrephus cabanisi*) free-ranging in the Taita Hills. © Tom Callens (A) and Alexander Callens (B)

P. cabanisi was deemed highly suitable as ecological model species to study genetic and demographic signatures of rainforest fragmentation, for the following reasons: (i) Due to long research history in the Taita Hills, an extensive bird ringing dataset was available at the start of the study (+3000 *P. cabanisi* captured and individually marked to date), allowing to quantify temporal and spatial variation in demographic and genetic processes. (ii) Levels of stress sensitivity inferred from spatiotemporal patterns in tarsus asymmetry, a proxy of environmental stress, indicated that the species was at least moderately sensitive to indigenous forest disturbance (Lens *et al.* 1999). (iii) *P. cabanisi* is a group-living, ant-following and insectivorous understory specialist with preliminary indications of cooperative breeding, displaying all characteristics to be highly vulnerable to extinction if small fragments suffer from severe isolation (Walters *et al.* 2004, Van Houtan *et al.* 2006, Fischer and Lindenmayer 2007, Sigel *et al.* 2010); (iv) Detection and monitoring of nests is highly feasible, rendering *P. cabanisi* a good candidate species to study spatial and temporal variation in predation rates.

Moreover, ecological insights of *P. cabanisi* may be used to guide conservation actions for ecologically similar but more endangered taxa, as accurate data collection is generally unfeasible for the latter species (Caro *et al.* 2005). To serve as a surrogate, a species must respond to relevant environmental conditions or disturbance in a way similar to the target species (Landres *et al.* 1988). In the Taita Hills, *P. cabanisi* may serve as a surrogate for critically endangered species such as *T. helleri* for the following reasons: (i) Both species are forest specialists, confined to the interior of undisturbed forest with breeding invariably occurring within forest boundaries (Bennun *et al.* 1996); (ii) Historical changes in levels of stress sensitivity showed identical responses in both taxa (Lens *et al.* 1999); (iii) Both species showed similar relationships between population levels of stress sensitivity and habitat disturbance (Lens *et al.* 1999); (iv) Patch occupancy seemed to be affected in identical ways due to forest fragmentation and dispersal limitations, with *P. cabanisi* occupying four and *T. helleri* only one of eight small indigenous forest remnants in the Taita Hills (Lens *et al.* 2002b); (v) Both species construct similar off-ground open-cup shaped nests (Keith *et al.* 1992), possibly attracting identical predators.

STUDY AREA

Field work was carried out in remnant indigenous forest patches within the Taita Hills of south-east Kenya (Fig. 1, 03°24'S, 38°21'E, altitude 1200-2220 m; Photo 4). This mountain range represents the northernmost extension of the Eastern Arc Mountains (EAM), a chain of mountains that runs from south-eastern Kenya to southern Tanzania (Lovett 1986, Lovett and Wasser 1993). It is an area of high conservation priority (Myers *et al.* 2000) and part of the Eastern Afromontane biodiversity hotspot (Mittermeier *et al.* 2005). The combined effect of long isolation (separation from the main Guineo-Congolian forests of west and central Africa by the Miocene uplifts of the central African plateau 10 to 17 million years ago) and climatic stability has boosted this region with a high diversity of fauna and flora combined with very high levels of endemism (Lovett and Wasser 1993). Indigenous forests of the EAM are currently scattered over ca. 5076 km² (Platts *et al.* 2010) with both rapid and drastic deforestation together with high levels of threat (Mittermeier *et al.* 1998, Newmark 1998, Stattersfield *et al.* 1998).

Within the globally important biodiversity hotspot of the EAM, the Taita Hills section ranks among the most threatened sites (Lovett and Wasser 1993, Burgess *et al.* 2007), with the total area of indigenous forest cover having decreased by ca. 50% since 1955 (Beentje 1987, Newmark 1998, Myers *et al.* 2000, Pellikka *et al.* 2009). Deforestation mainly resulted from clearance for timber and agriculture (Bennun and Njoroge 1999, Myers *et al.* 2000). Additionally, remaining forests have been used for encroachment agriculture, firewood collection and grazing (Wilder *et al.* 1998, Morara 2005). While large-scale timber extraction and encroachment agriculture have been stopped to a great extent, a considerable degradation of some indigenous forests from firewood collection and grazing is still occurring (Githiru *et al.* 2011). Even though half of the indigenous forest cover has been lost, the total forest area remained nearly equal due to the large-scale planting of exotic plantations (Beentje 1987) such as cypress (*Cupressus lusitanica*), eucalyptus (*Eucalyptus saligna*), pines (*Pinus elliottii, P. caribea,* and *P. patula*), *Maesopsis eminii* and grevillea (*Grevillea robusta*) (Pellikka *et al.* 2009). The balance between exotic and indigenous forest might explain why the Taita forests continue to harbor a highly diverse fauna and flora, with 13 endemic plant(sub)species (Beentje 1987) and two endemic bird species. The two endemic bird species are the Taita apalis (*Apalis fuscigularis*) and the Taita thrush (*Turdus helleri*) (Brooks *et al.* 1998) which

are both listed as 'Critically Endangered' (Birdlife International 2012), justifying the classification of the Taita Hills as an Important Bird Area (Bennun and Njoroge 1999).

At present, the Taita forest archipelago covers an area of ca. 430 ha of indigenous forest dominated by tree species *Tabernaemontana stapfiana, Albizia gummifera, Macaranga conglomerata, Strombosia scheffleri, Newtonia buchananii* and *Xymalos monospora* (Chege and Bytebier 2005, Rogers *et al.* 2008, Aerts *et al.* 2011). These mountain blocks are isolated from other highlands blocks by over 80 km of semi-arid plains in either direction (Lovett 1985, Pellikka *et al.* 2009). The few remaining indigenous forests are mainly located at hilltops and ridges and are isolated by small holder cultivation plots, bushes and exotic plantations (Beentje 1987, Adriaensen *et al.* 2006, Pellikka *et al.* 2009). Three larger (86-220 ha) and eight small (2-8 ha) forest fragments are located on two mountain isolates. Dabida isolate (9 fragments, including the highest peak of the Taita Hills at 2228 m) is separated by the low-altitude 'Paranga' valley from the nearby Mbololo isolate (2 fragments) (Fig. 2, Brooks *et al.* 1998, Pellikka *et al.* 2009).

Photo 4. Overview of the Taita Hills' landscape, taken from Chawia forest. © Tom Callens

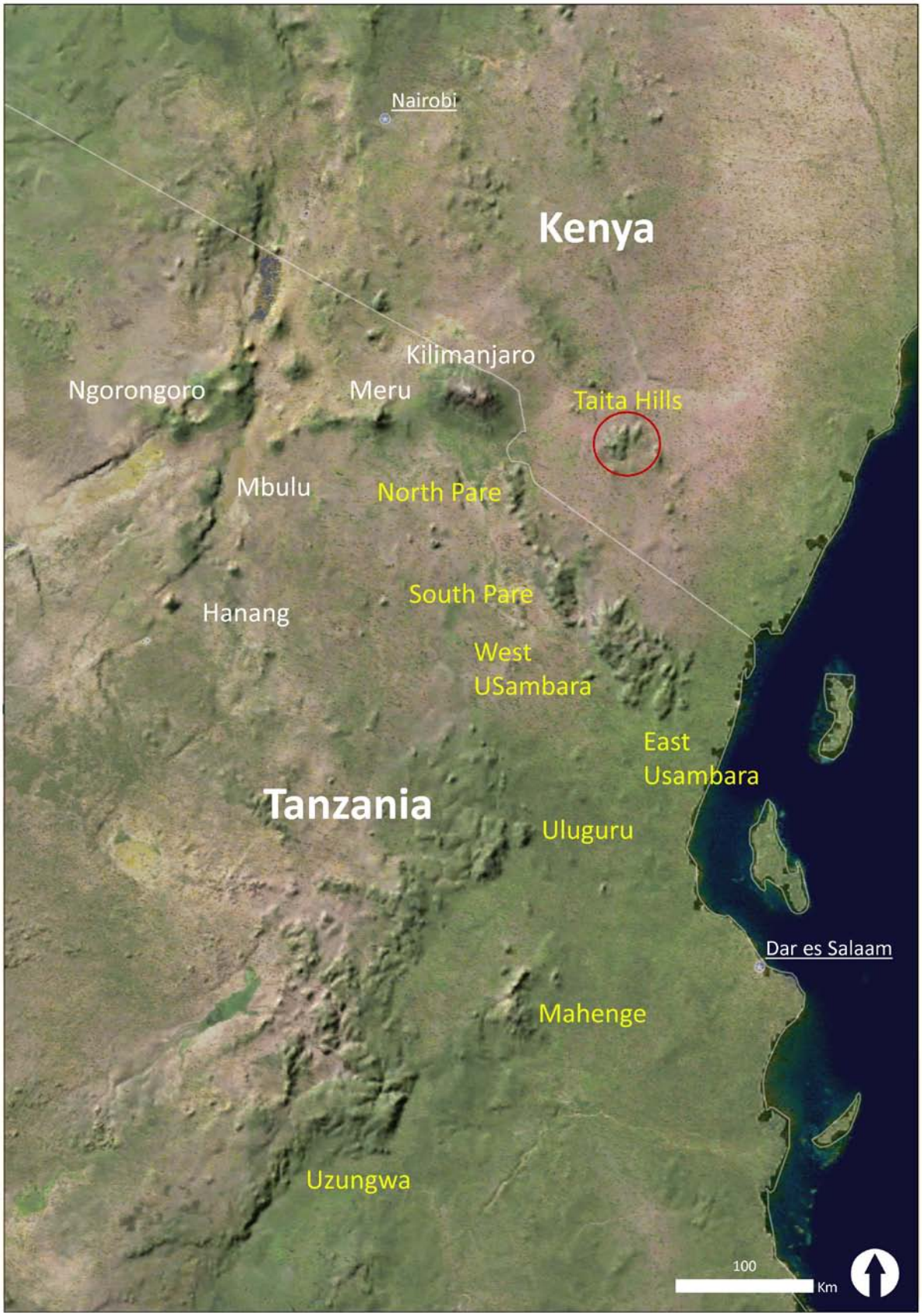

Figure 1: Location of the Taita Hills relative to other forested areas (highlighted in yellow) within the Eastern Arc Mountain (EAM) biodiversity hotspot (© Worldmap from ESRI Data & Maps, 2011).

Data collection for this study was restricted to seven indigenous forest fragments located on these two mountain isolates (Table 1, areas of indigenous forest; Table 2, pairwise distance matrix for all studied forest fragments), in which nesting behavior by *P. cabanisi* had been recorded and from which long-term ringing data (since 1996) were available. Four small forest fragments [Fururu (FU, ca. 8 ha), Ndiwenyi (ND, ca. 4 ha), Mwachora (MW, ca. 2.5 ha), Macha (MA, ca. 2.3 ha)] are all located on the Dabida isolate, and are strongly disturbed both in terms of vegetation structure and composition (Chege and Bytebier 2005). Mbololo forest (MB, ca. 185 ha, Mbololo isolate; Photo 5A) comprises the largest and most pristine fragment (Brooks *et al.* 1998, Lens *et al.* 1999, Omoro *et al.* 2010) and constitutes the population stronghold of the Taita thrush *Turdus helleri* (Lens *et al.* 2002a). Ngangao (NG, ca. 120 ha, Dabida isolate) is the second-largest fragment. It is labeled as "intermediately degraded", with past pit-sawing activities but only minor evidence of ongoing human disturbance (Brooks *et al.* 1998, Lens *et al.* 1999, Omoro *et al.* 2010, Borghesio *et al.* 2010). Chawia (CH, ca. 86 ha, Dabida isolate; Photo 5B) is a medium-sized forest that shows the strongest signature of anthropogenic disturbance among the three largest fragments. Past and present forest deterioration is mainly related to agricultural activities, firewood collection, charcoal production (Beentje 1987), clearing for the establishment of exotic stands (Pellikka *et al.* 2009), cattle grazing, logging and pole cutting (Wilder *et al.* 1998, Lens *et al.* 1999, Chege and Bytebier 2005, Githiru *et al.* 2005a; Photo 6A&B). In the past, these activities nearly resulted in extinction of a local subpopulation of the critically-endangered Taita thrush (*Turdus helleri*) (Galbusera *et al.* 2000).

Photo 5. Indigenous cloud forest in Mbololo (A) and Chawia (B) in the Taita Hills. © Tom Callens

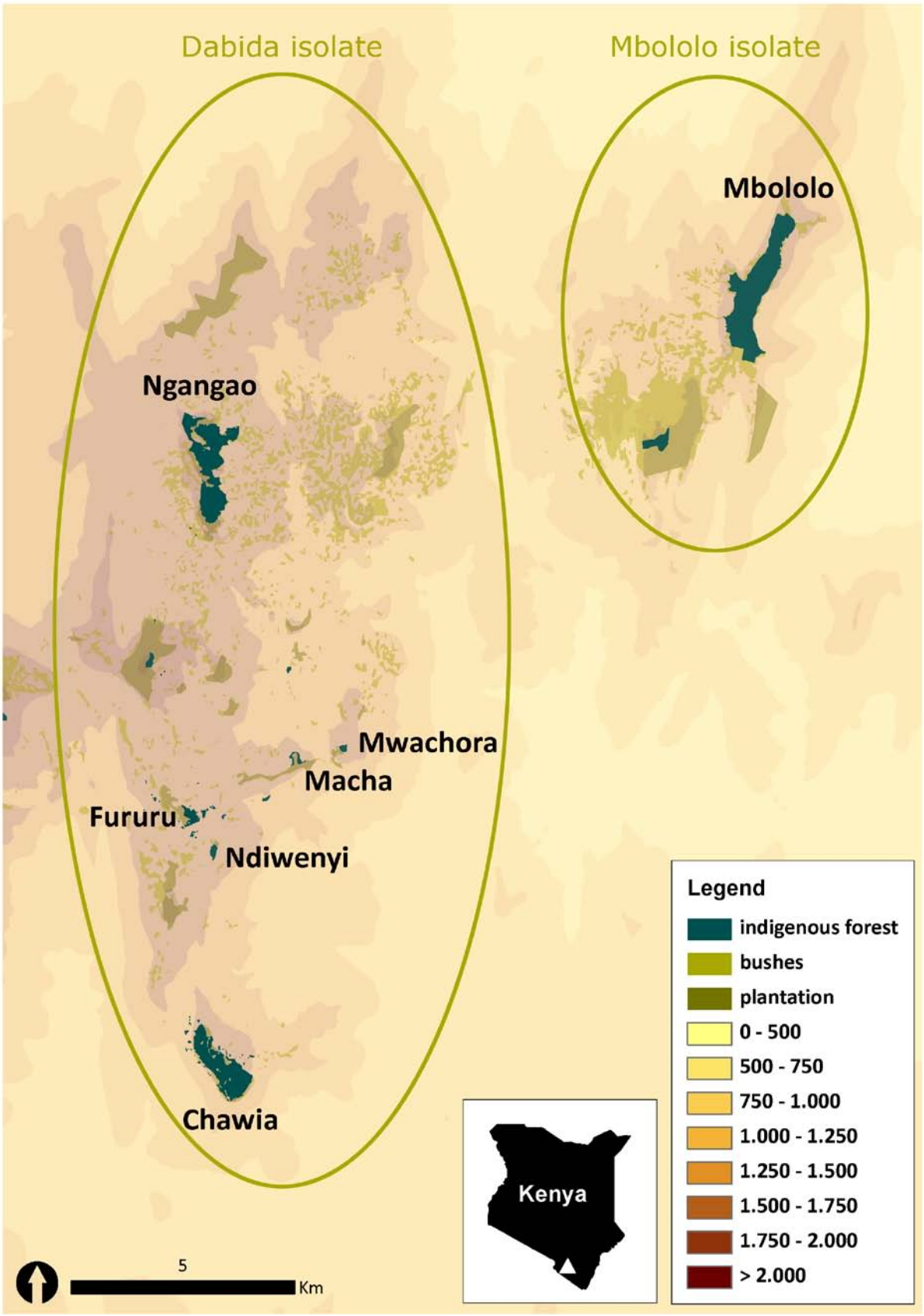

Figure 2: Map depicting all remaining indigenous forest fragments in the Taita Hills (SE Kenya), indicating the location of three large (Mbololo, Chawia, Ngangao) and two small (Fururu, Ndiwenyi) fragments that hold established breeding populations of *P. cabanisi*. Two other tiny forest remnants (Macha, Mwachora) were only colonized in the recent past. The two mountain isolates (Dabida and Mbololo) are indicated with circles.

Table 1. Overview of Taita cloud forest fragments and data structure in the five PhD chapters. Fragments with recorded breeding activity of *P. cabanisi* populations are highlighted in bold. Area estimates are based on airborne digital camera imagery (25.01.2004, Pellikka *et al.* 2009) or GPS readings (Githiru 2003). Altitudinal range of the indigenous forest cover is generated from the Digital Elevation Model of the Taita Hills (Taita Hills Biodiversity Project 2001).

Isolate	Forest fragment	Abbreviation	Indigenous forest (in ha)	Altitudinal range (m) of the indigenous forest	Chapter				
					1	2	3	4	5
Mbololo	**Mbololo**	MB	185	1425 – 1775	x	x			x
	Ronge	RO	4	1281 – 1381					
Dabida	**Ngangao**	NG	120	1641 – 1942	x	x	x	x	x
	Chawia	CH	86	1496 – 1616	x	x	x	x	x
	Yale	YA	15	1703 – 2078					
	Fururu	FU	8	1625 – 1723		x			x
	Ndiwenyi	ND	4	1590 – 1604		x			x
	Macha	MA	2.3	1570 – 1658		x			
	Mwachora	MW	2.5	1625 – 1656		x			
	Vuria	VU	2	1875 – 2014					
	Kichuchenyi	KI	1	1447 – 1507					

Photo 6. Anthropogenic disturbance in the Taita Hills: firewood collection (A) and cutting down indigenous trees in Chawia forest (B). © Tom Callens

Table 2. Pairwise distances (in km) between seven indigenous forest fragments of the Taita Hills in SE Kenya. Estimates are based on forest boundary vector layers from recent aerial photographs (25.01.2004, Pellikka *et al.* 2009) and a SPOT 4 image (15.10.2003, Clark and Pellikka 2009). Abbreviations are adopted from Table 1.

	MB	NG	CH	FU	ND	MA
NG	11					
CH	19	11				
FU	16	6	4			
ND	16	7	4	1		
MA	13	5	6	2	2	
MW	12	6	7	3	3	1

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Seven sympatric, forest-dependent bird species from the Taita Hills that range from being highly mobile (up) to highly sedentary (down): (A) Olive sunbird *Nectarinia olivacea* (© Tom Callens), (B) Taita white-eye *Zosterops (poliogastrus) silvanus* (© Tom Callens), (C) Stripe-cheeked greenbul *Andropadus milanjensis striifacies* (© Tom Callens), (D) White-starred robin *Pogonocichla stellata* (© THBP), (E) Cabanis's greenbul *Phyllastrephus cabanisi placidus* (© Tom Callens), (F) Taita thursh *Turdus helleri* (© THBP), and (G) Yellow-throated woodland warbler *Phylloscopus ruficapilla* (© David Gitau).

Genetic signature of population fragmentation varies with mobility in seven bird species of a fragmented Kenyan cloud forest

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ABSTRACT

Habitat fragmentation can restrict gene flow, reduce neighbourhood effective population size, and increase genetic drift and inbreeding in small, isolated habitat remnants. The extent to which habitat fragmentation leads to population fragmentation, however, differs among landscapes and taxa. Commonly, researchers use information on the current status of a species to predict population effects of habitat fragmentation. Such methods, however, do not convey information on species-specific responses to fragmentation. Here we compare levels of past population differentiation, estimated from microsatellite genotypes, with contemporary dispersal rates, estimated from multi-strata capture-recapture models, to infer changes in mobility over time in seven sympatric, forest-dependent bird species of a Kenyan cloud forest archipelago. Overall, populations of sedentary species were more strongly differentiated and clustered compared to those of vagile ones, while geographic patterning suggested an important role of landscape structure in shaping genetic variation. However, five of seven species with broadly similar levels of genetic differentiation nevertheless differed substantially in their current dispersal rates. We conclude that post-fragmentation levels of vagility, without reference to past population connectivity, may not be the best predictor of how forest fragmentation affects the life history of forest-dependent species. As effective conservation strategies often hinge on accurate prediction of shifts in ecological and genetic relationships among populations, conservation practices based solely upon current population abundances or movements may, in the long term, prove to be inadequate.

INTRODUCTION

Habitat loss and fragmentation are considered key drivers of biodiversity loss (Turner 1996), in particular in historically stable, species-rich ecosystems such as tropical rainforests (Laurance *et al.* 2002; Waltert *et al.* 2005; Kirika *et al.* 2008). While habitat fragmentation may affect population dynamics in diverse and complex ways (Lewis 2006), long-term viability of species often hinges on their genetic population structure (Frankham *et al.* 2002). Habitat fragmentation can affect the genetic structure of populations both directly and indirectly, i.e. by restricting gene flow, reducing neighbourhood effective population sizes and/or increasing the levels of genetic drift and inbreeding in small, isolated habitat remnants (reviewed in Frankham *et al.* 2002). The extent to which habitat fragmentation leads to population fragmentation, however, depends on properties of the landscapes in which suitable habitat patches are embedded, and of the taxa that depend on these patches for their survival or reproduction (Caizergues *et al.* 2003). Along these lines, levels of genetic differentiation are generally lower in more connected landscapes, defined as the degree to which landscape elements facilitate movements between resource patches (Taylor *et al.* 1993), and in taxa that can cross hostile habitats more easily, such as birds (Crochet 2000; Ehrich *et al.* 2001; Ehrich & Stenseth 2001; Goossens *et al.* 2001). Because birds are considered a highly vagile group able to transverse wide spans of unsuitable habitat, their degree of within-species genetic structuring has been underappreciated in the past, especially so in montane species and tropical forest specialists (e.g. Brown *et al.* 2004; Moore *et al.* 2008 and references therein). However, a number of avian genetic studies did show high levels of population differentiation over small spatial scales, either as a result of severe philopatry, narrow habitat requirements or a ground-dwelling lifestyle with restricted flight ability (Avise 1996; Brown *et al.* 2004).

When key habitat of sedentary or specialist species becomes progressively fragmented, dispersal among remnant subpopulations may become disrupted and, in turn, affect population viability and rates of local adaptation (Hanski & Gilpin 1991). Dispersal and gene flow therefore comprise key processes underlying the regulation, persistence and adaptive evolution of spatially-structured populations in heterogeneous landscapes (Slatkin 1987; Hanski & Gilpin 1991; Frankham 1997; Hanski 1998; Whitlock & McCauley 1999). However, not all species appear to be equally sensitive to fragmentation (Van Houtan *et al.* 2006, 2007; Sekercioglu 2007), so it is of high importance to determine which species are more prone to extinction to improve future conservation actions. Commonly, researchers use

information on the current status of a species (e.g. patch occupancy, current dispersal; Lens *et al.* 2002), but these methods do not imply any information on the species-specific response to fragmentation. For example, a species can have built up an "extinction debt", which can result into a higher vulnerability for future extinctions (Tilman *et al.* 1994; Hanski & Ovaskainen 2002; Bulman *et al.* 2007), while other species might be more buffered from the loss of genetic diversity caused by fragmentation (Howeth *et al.* 2008). An alternative approach, therefore, is to infer species-specific responses to habitat fragmentation from genetic data which can provide information on historical processes prior to the date of sampling (Wilmer & Wilcox 2007; Lada *et al.* 2008; Howeth *et al.* 2008; Oddou-Muratorio & Klein 2008; Pavlacky *et al.* 2009). Genetic differentiation among populations is traditionally measured by Wright's F_{ST} (1931) or its derivates G_{ST}, G_{ST}', and D_{est} (Hedrick 2005b, Hedgecock *et al.* 2007, Jost 2008, Waples *et al.* 2008). These estimates are considered to reflect historical rates of gene flow integrated over multiple generations (Allendorf & Luikart 2007), although gene flow estimates can be biased in case of mutation-drift (Hedrick 2005b) or migration-drift (Whitlock & McCauley 1999, Lowe and Allendorf 2010) disequilibrium. Genetic estimates that indirectly reflect past population connectivity may be particularly informative when combined with direct estimates of contemporary dispersal rates among a restricted number of focal populations (Koenig *et al.* 1996, Whitlock & McCauley 1999, Faubet *et al.* 2007), thereby allowing an evaluation of changes in mobility over time (Howeth *et al.* 2008; Lowe & Allendorf 2010).

Here we report on a study of the genetic signature of population fragmentation in seven sympatric, forest-dependent bird species of a Kenyan cloud forest archipelago that were earlier shown to differ in their response to forest fragmentation, as inferred from post-fragmentation levels of mobility and patch occupancy (Lens *et al.* 2002). A diverse set of genetic parameters/tests were used to infer signals of bottlenecks (deviation from mutation/drift equilibrium) and changes in gene flow over time (migration/drift equilibrium). We compare species-specific levels of genetic population differentiation using D_{est} (Jost 2008), representing historic dispersal rates averaged over time (Bohonak 1999; Allendorf & Luikart 2007; Palsboll *et al.* 2007), and compare these with contemporary dispersal rates based on multi-strata mark-recapture analyses (data from Lens *et al.* 2002). Ultimately we assess whether the magnitude of genetic effects is correlated with loss of mobility.

MATERIALS AND METHODS

Study area and species

The Taita Hills (SE Kenya, 03°24'S, 38°21'E) represent the northernmost part of the Eastern Arc Mountains biodiversity hotspot of Kenya and Tanzania (Lovett and Wasser 1993). They cover an area of ca 250 km² and are isolated from other highlands by over 80 km of semi-arid plains in either direction (Lovett 1985, Pellikka *et al.* 2009). Since 1955, indigenous forest cover in the Taita Hills decreased by over 50%, and forest remnants are mainly located at hilltops and ridges and isolated by small holder cultivation plots and exotic plantation forests (Lens *et al.* 1999; Adriaensen *et al.* 2006; Pellikka *et al.* 2009). Three larger forest fragments (86-185 ha) and eight small ones (2-8 ha) are located on two mountain isolates (Dabida: 9 fragments; Mbololo: 2 fragments) separated by a low-altitude valley (Fig. 2 General Introduction; Brooks *et al.* 1998; Pellikka *et al.* 2009). Smaller fragments are most heavily disturbed, mainly as a result of logging, pole cutting and cattle grazing (Chege & Bytebier 2005). Among the three largest fragments, Chawia forest (CH, 86 ha; Dabida isolate) is most heavily degraded, Ngangao forest (NG, 120 ha; Dabida isolate) is intermediately degraded, and Mbololo forest (MB, 185 ha; Mbololo isolate) is most pristine (Brooks *et al.* 1998; Lens *et al.* 1999). The three largest fragments are inhabited by all seven study species, whereas the cluster of smaller fragments hosts subsets of these species only (based on breeding evidence during 1996-2001 in Lens *et al.* 2002), and analyses in this study are restricted to breeding populations in fragments CH, NG and MB.

Between 1996 and 2009, a total of 5002 individuals of seven forest-dependent species were trapped (no use of tape luring or artificial feeders), marked, measured and blood-sampled in fragments CH, NG and MB: Stripe-cheeked greenbul (*Andropadus milanjensis striifacies*; 626 ind), cabanis's greenbul (*Phyllastrephus cabanisi placidus*; 679 ind), Taita thrush (*Turdus helleri*; 491 ind), white-starred robin (*Pogonocichla stellata helleri*; 2262 ind), Taita white-eye (*Zosterops (poliogastrus) silvanus*; 323 ind), olive sunbird (*Nectarinia olivacea changamwensis*; 493 ind), and yellow-throated wood-warbler (*Phylloscopus ruficapilla minullus*; 128 ind). *P. ruficapilla, T. helleri, A. milanjensis, P. cabanisi* and *N. olivacea* are presumed forest specialists, while *Z. silvanus* and *P. stellata* are presumed forest generalists (Bennun *et al.* 1996).

DNA extraction and genotyping

Upon capture of an individual, we collected 2-3 µl of blood from its brachial vein and stored it in 95% ethanol or DMSO. DNA was isolated either by boiling in a 5% Chelex solution (Biorad) after an incubation period of 90 min at 55°C in the presence of 100 µg proteinase K (ethanol storage) (Walsh *et al.* 1991) or by a normal phenol-chloroform extraction in the presence of 100 µg proteinase K (DMSO storage) (details in Galbusera *et al.* 2000). PCR amplification was executed in a 10 µl reaction volume containing approximately 100 ng DNA, 1x buffer (75 mM Tris-HCl pH 9.0, 20 mM $(NH_4)_2SO_4$, 0.01% Tween 20), 0.5 U Taq polymerase (Eurogentec), 200 µM dNTPs (GIBCO), 1.0-3.0 mM $MgCl_2$ (product size ranges and optimal reaction conditions in Appendix 1.A) and 250-500 nM of each primer from 4-10 variable microsatellite markers. Starting from the original PCR conditions, variable $MgCl_2$, template DNA concentrations and annealing temperatures were tested in a gradient PCR device (PC-960G Gradient Thermal Cycler, Labotechnic). Genotypes were scored on a 6% acrylamide gel in an automatic sequencer (ALF express, Pharmacia Biotech) or on an ABI 3130 Genetic Analyzer (Applied Biosystems) and microsatellite lengths were determined with GENEMAPPER Software 4.1. Microsatellite data for all species have been deposited in the Dryad data repository (doi:10.5061/dryad.8054).

Genetic analysis

Presence of null alleles was tested with program MICROCHECKER 2.2.3 by running 10000 Monte Carlo simulations and calculating 95% confidence intervals (Van Oosterhout *et al.* 2006). Deviation from Hardy–Weinberg equilibrium and linkage disequilibrium per locus were tested with program GENEPOP 4.0 (Raymond & Rousset 1995; Rousset 2008). Apart from the following marker*population combinations, all loci were consistent with Hardy-Weinberg equilibrium (all $P > 0.05$ after Bonferroni correction, Rice 1989): *P. stellata*: WBSW9 ($P = 0.0016$) and Pat14 in population CH ($P = 0.0019$); GF5B in population MB and NG ($P < 0.002$). Apart from WBSW9 and GF5B in *P. stellata*, none of the loci showed null alleles (removing both loci did not change our results; see also Galbusera *et al.* 2004). No linkage disequilibrium was detected between any pair of loci after correction for multiple testing (Rice 1989).

Observed and expected levels of heterozygosity were calculated with GENALEX 6.4 (Peakall & Smouse 2006), while allelic richness corrected for sample size was calculated with FSTAT 2.9.3.2 (Goudet 1995). To infer levels of population genetic differentiation, we calculated

overall and pairwise D_{est} values across all loci with SMOGD 1.2.5 (Crawford 2009). This estimate is increasingly considered more accurate than traditional ones such as F_{ST} (Wright 1951, 1965) and G_{ST} (Nei 1973; Nei & Chesser 1983) in accounting for differences in allelic diversity, especially for highly polymorphic microsatellite markers and in cases where assumptions for traditional calculations (e.g. migration/drift equilibrium) are violated (Jost 2008). For the sake of comparison, we also presented F_{ST} values (Wright 1951, 1965) calculated with GENEPOP 4.0 using parameter θ (Weir & Cockerham 1984; Raymond & Rousset 1995; Rousset 2008). To test whether relationships between genetic population differentiation and contemporary dispersal rates differed between fragments located on the same mountain isolate (NG-CH) or two different isolates separated by a dry valley (MB-NG, MB-CH), we performed an analysis of variance in SAS 9.2 (SAS Institute, 2002-2008).

The genetic population structure of each species was inferred from a Bayesian admixture model implemented in TESS 2.3. This procedure earlier proved to be more powerful than non-spatial algorithms, especially in weakly differentiated populations (Chen *et al.* 2007; Durand *et al.* 2009). To properly assess the genetic population structure of each species, we explored a wide range of values for the number of genetic clusters, K (varying from 1 to 9), and assessed the fit of the model to the data for each value (Francois & Durand 2010). A total of 100 independent iterations (each 50000 sweeps long and discarding the first 30000 sweeps) were run for each value of K. Model fits were compared with the Deviance Information Criterion (DIC, Spiegelhalter *et al.* 2002), a penalized measure of fit accounting for model complexity (models with lower DIC values fit data better). DIC values averaged over 100 independent iterations were plotted against K, and K-values for which DIC values first reached a plateau were selected (procedure similar to 'logarithm of evidence' in STRUCTURE; Evanno *et al.* 2005). The 10 runs with lowest DIC values for the selected K-value were retained and their admixture estimates were averaged using CLUMPP version 1.1.1 (Jakobsson & Rosenberg 2007), applying the greedy algorithm with random input order and 1000 permutations to align the runs and calculate G' statistics. Results were visualized using DISTRUCT 1.1 (Rosenberg 2004).

Deviation from mutation/drift equilibrium was tested by comparing levels of heterozygosity calculated from observed allele frequencies (*sensu* Nei *et al.* 1975, Nei 1987) with those expected under equilibrium with program BOTTLENECK 1.2.02 (Piry *et al.* 1999). As the mutation model underlying our microsatellite markers was unknown, data were analyzed

under two different model assumptions: two-phase model (TPM) and stepwise mutation model (SMM) (Luikart & Cornuet 1998; Di Rienzo *et al.* 1994; Jarne & Lagoda 1996; Piry *et al.* 1999). When modeling TPM models, combinations of 95% single-step mutations and 5% multistep mutations were used, with a variance of 30 among multiple-step mutations (10^4 replications) (Piry *et al.* 1999).

Deviation from migration/drift equilibrium was tested by comparing the relative likelihoods of 'gene flow/drift' and 'drift only' models with the program 2MOD, using a MCMC procedure with 10^5 iterations and a burn-in of 10^4 (Ciofi *et al.* 1999). Time intervals between population founding and population sampling were assumed sufficiently short such that effects of mutations were negligible (drift only), while mutation rates were assumed much smaller than migration rates (gene flow/drift). Data were analyzed under both models and Bayes factors were calculated to infer decisive power estimates of the most likely models (Jeffreys 1961; Goodman 1999).

Contemporary dispersal rates

Contemporary dispersal rates were available for each of the seven study species from Lens *et al.* (2002), based on capture-recapture histories of 3089 individuals trapped and individually marked in the large fragments CH, NG, and MB between 1996 and 2002. Individual recapture histories (based on mist net recaptures and color-ring observations) were generated using time intervals of 1 month. A total of 889 individuals were captured-recaptured in two or more months, and among these, 47 individuals were captured-recaptured in two or more fragments. Monthly probabilities of between-fragment dispersal were estimated from multistrata mark-recapture models in program MARK (White & Burnham 1999). Based on Akaike's Information Criteria (AIC), the most parsimonious model was selected from a candidate set in which monthly probabilities of survival, recapture, and dispersal were modeled simultaneously. Because distances differed between pairs of fragments (MB-NG, 11.3 km; MB-CH, 19.4 km; NG-CH, 10.9 km) and estimates of dispersal might be biased by differences in abundance between fragments for the different species, multistrata models were constructed that estimated monthly dispersal probabilities between each pair of fragments irrespective of the direction of dispersal. In a final step, we regressed pairwise dispersal probabilities against pairwise distances between the three forest fragments for each species, then used the intercept of each regression equation as the species-specific measure of contemporary dispersal (see Lens *et al*. 2002 for details on candidate models). Contemporary

dispersal rates were positively correlated with contemporary gene flow rates estimated with BAYESASS 1.3 (Wilson & Rannala 2003) ($F_{1,5} = 9.93$, $P = 0.025$). Species-specific estimates were based on 3000000 iterations, a burn-in of 999999, a sampling frequency of 2000, and a delta–value of 0.15. Because of the large confidence intervals (probably resulting from the low number of individuals and markers compared to those recommended by Wilson & Rannala 2003) and the fact that gene flow estimates are difficult to interpret when levels of genetic differentiation are low (Faubet *et al.* 2007), contemporary mobility was inferred from rates of dispersal, rather than gene flow, that were based on larger datasets, multiple recapture events, and multiple years.

RESULTS

Genetic population structure

Genetic differentiation was highly variable among the seven study species, as shown by strong differences in both pairwise (between populations CH, NG and MB) and overall estimates of D_{est} (Table 1.1, see F_{ST} values for comparison). D_{est} estimates were very low (close to 0) in *N. olivacea*, markedly high (0.2 to 0.4) in *T. helleri*, and intermediate in the remaining species. Figure 1.1 depicts the level of genetic clustering for each study species with barplots per individual visualizing the inferred admixture proportions within each fragment. Individuals of *T. helleri*, *P. ruficapilla* and *P. cabanisi* were assigned to three genetic clusters corresponding to the three forest fragments (see Appendix 1.B for details on model choice). Cluster differentiation was strong for *T. helleri* and *P. ruficapilla* but lower for *P. cabanisi* (Fig. 1.1). Individuals of *A. milanjenis*, *P. stellata* and *Z. silvanus* were assigned to two genetic clusters, in each case with one cluster consisting of populations CH and NG, and the other consisting of population MB, with the highest differentiation in *A. milanjensis* and the lowest differentiation in *P. stellata* (Fig. 1.1). Individuals of *N. olivacea* were assigned to a single panmictic cluster. Overall, strong genetic clustering corresponded with strong genetic differentiation (high D_{est}) and vice versa (Fig. 1.1, Table 1.1). In all species except for *P. cabanisi*, pairwise genetic differentiation between populations CH-NG, located on the same isolate, was consistently lower than between populations CH-MB and NG-MB, located on two different isolates ($F_{1,11.1} = 7.48$; $P = 0.019$; Fig. 2 General Introduction, Table 1.1).

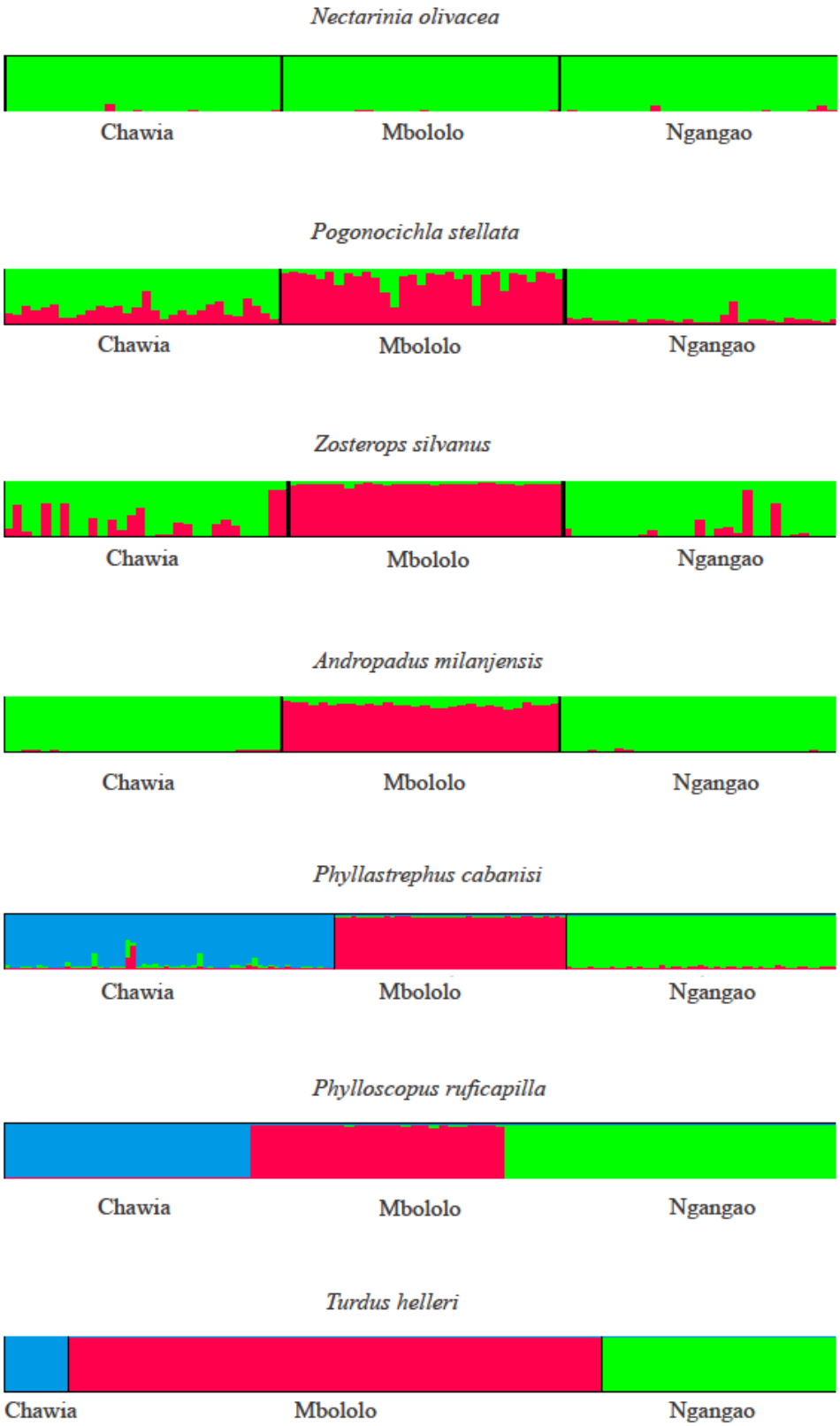

Figure 1.1. Admixture proportions for seven bird species of the Taita Hills (SE Kenya) as inferred from Bayesian genetic clustering. Each bar represents an individual sample with color codes reflecting the likelihood of belonging to one of the inferred genetic clusters.

Table 1.1. Levels of genetic differentiation across loci between populations Chawia (CH), Mbololo (MB) and Ngangao (NG) as inferred from overall D_{est} and F_{ST} values (bold font) and harmonic means of the pairwise values (regular font), for seven bird species of the Taita Hills (SE Kenya).

	D_{est}			F_{ST}		
	CH	MB	Overall	CH	MB	Overall
Nectarinia olivacea						
MB	0.002			0.009		
NG	0.00003	0.0007	**0.001**	0.001	0.006	**0.006**
Pogonocichla. stellata						
MB	0.029			0.025		
NG	0.015	0.054	**0.034**	0.008	0.039	**0.024**
Zosterops silvanus						
MB	0.057			0.049		
NG	0.022	0.086	**0.064**	0.019	0.065	**0.044**
Andropadus milanjensis						
MB	0.049			0.114		
NG	0.002	0.055	**0.036**	0.010	0.096	**0.076**
Phyllastrephus cabanisi						
MB	0.054			0.040		
NG	0.044	0.041	**0.058**	0.043	0.040	**0.042**
Phylloscopus ruficapilla						
MB	0.048			0.083		
NG	0.021	0.062	**0.058**	0.066	0.133	**0.095**
Turdus helleri						
MB	0.422			0.250		
NG	0.204	0.362	**0.356**	0.113	0.207	**0.207**

Genetic equilibria and bottlenecks

Levels of allelic richness and observed and expected heterozygosity did not significantly differ among populations (Non-parametric Friedman test: all Fr between 1.556-4.571; all $P > 0.05$; see values in Table 1.2). Values of allelic richness and H_O in populations MB and NG of *P. ruficapilla* were low compared to all other species. However, due to large variability in number and type of microsatellite markers among species, this difference could not be tested statistically. Levels of heterozygosity significantly exceeded those expected under mutation-drift equilibrium (Table 1.3) in population CH of species *T. helleri* (Wilcoxon test; TPM: $P = 0.008$; SMM: $P = 0.016$), populations MB and NG of *P. ruficapilla* (MB: TPM and SMM: $P = 0.02$; NG: TPM and SMM: $P = 0.008$) and population NG of *Z. silvanus* (TPM: $P = 0.03$; not significant after clustering with population CH; Appendix 1.C). None of the other populations or clusters showed evidence of heterozygosity excess. In *P. ruficapilla*, 'drift only' models were 16.8 times more likely than 'migration/drift equilibrium' models (Table

1.4). In all other species, 'migration/drift' models were more likely than pure drift models, whereby evidence for migration/drift equilibrium ranged from decisive to substantial in *T. helleri, P. stellata, A. milanjensis, N. olivacea,* but was weak in *Z. silvanus* and *P. cabanisi*.

Table 1.2. Population-level allelic richness and heterozygosity in seven bird species of the Taita Hills (SE Kenya) with N = number of original samples; loci = number of loci; A_R = allelic richness corrected for sample size (number of samples between brackets); H_O= observed heterozygosity; H_E = expected heterozygosity.

Population	Species	*N*	Loci	A_R	H_O	H_E
Chawia	*Nectarinia olivacea*	30	8	4.1 (30)	0.48	0.50
	Pogonocichla stellata	30	7	8.7 (30)	0.65	0.71
	Zosterops silvanus	30	4	5.5 (29)	0.53	0.59
	Andropadus milanjensis	30	7	3.1 (30)	0.41	0.39
	Phyllastrephus cabanisi	60	10	6.0 (42)	0.64	0.61
	Phylloscopus ruficapilla	26	7	3.3 (26)	0.56	0.52
	Turdus helleri	18	6	4.0 (18)	0.68	0.62
Mbololo	*Nectarinia olivacea*	30	8	4.0 (30)	0.51	0.49
	Pogonocichla stellata	31	7	7.2 (30)	0.57	0.64
	Zosterops silvanus	29	4	4.0 (29)	0.49	0.51
	Andropadus milanjensis	30	7	3.3 (30)	0.45	0.44
	Phyllastrephus cabanisi	42	10	5.4 (42)	0.63	0.62
	Phylloscopus ruficapilla	27	7	2.6 (26)	0.44	0.44
	Turdus helleri	152	6	4.5 (18)	0.59	0.59
Ngangao	*Nectarinia olivacea*	30	8	4.2 (30)	0.49	0.47
	Pogonocichla stellata	30	7	7.4 (30)	0.60	0.67
	Zosterops silvanus	29	4	6.0 (29)	0.66	0.65
	Andropadus milanjensis	30	7	3.4 (30)	0.46	0.44
	Phyllastrephus cabanisi	49	10	6.4 (42)	0.61	0.58
	Phylloscopus ruficapilla	35	7	2.3 (26)	0.45	0.42
	Turdus helleri	67	6	5.4 (18)	0.67	0.66

Table 1.3. Population-level excess of heterozygote genotypes in 3 populations of seven bird species of the Taita Hills (SE Kenya) with CH = Chawia; MB = Mbololo; NG = Ngangao. Wilcoxon-based levels of significance are shown for different mutation models ($P < 0.05$ indicated in bold).

Species	Population	TPM[1]	SMM[2]
Nectarinia olivacea	CH	0.680	0.844
	MB	0.629	0.727
	NG	0.809	0.902
Pogonocichla stellata	CH	0.594	0.973
	MB	0.992	1.000
	NG	0.766	0.973
Zosterops silvanus	CH	0.438	0.938
	MB	0.156	0.156
	NG	**0.031**	0.563
Andropadus milanjensis	CH	0.711	0.766
	MB	0.594	0.656
	NG	0.656	0.711
Phyllastrephus cabanisi	CH	0.754	0.862
	MB	0.246	0.313
	NG	0.862	0.947
Phylloscopus ruficapilla	CH	0.148	0.148
	MB	**0.020**	**0.020**
	NG	**0.008**	**0.008**
Turdus helleri	CH	**0.008**	**0.016**
	MB	0.781	0.977
	NG	0.578	0.922

[1]Two-Phase Model; [2]Stepwise Mutation Model

Table 1.4. Species-level likelihood of 'migration/drift equilibrium' and 'drift only' models. Bayes factors are calculated as ratios of most to least likely models and translated into levels of decisive power (following Jeffreys (1961)). The single species that shows strong evidence for migration-drift disequilibrium is indicated in bold.

Species	Migration-drift equilibrium model	Drift only model	Bayes Factor	
Phylloscopus ruficapilla	**1123**	**18877**	**16.8**	**(Strong)**
Turdus helleri	19945	55	362.6	(Decisive)
Pogonocichla stellata	19446	554	35.7	(Very strong)
Andropadus milanjensis	17514	2486	7.0	(Substantial)
Nectarinia olivacea	15101	4899	3.1	(Substantial)
Zosterops silvanus	13311	6689	2.0	(Weak)
Phyllastrephus cabanisi	11490	8510	1.4	(Weak)

Change in mobility over time

Figure 1.2 relates species-specific levels of genetic population differentiation and patch occupancy to contemporary dispersal rates. *N. olivacea* showed the lowest level of genetic differentiation, the highest levels of patch occupancy and contemporary dispersal, and a single genetic cluster. *T. helleri*, in contrast, showed very strong genetic differentiation, very low levels of patch occupancy and contemporary dispersal, and three genetic clusters. The other five species were distinguished by broadly similar levels of genetic differentiation and two or three genetic clusters, but varied strongly in contemporary dispersal (ranging from zero in *P. ruficapilla* to relatively high in *Z. silvanus*) and patch occupancy (3-8 forest fragments occupied). Relationships between genetic differentiation and contemporary dispersal did not differ when fragments were located on the same or different mountain isolates ($F_{1,11.2}$=1.04, *P* = 0.3286).

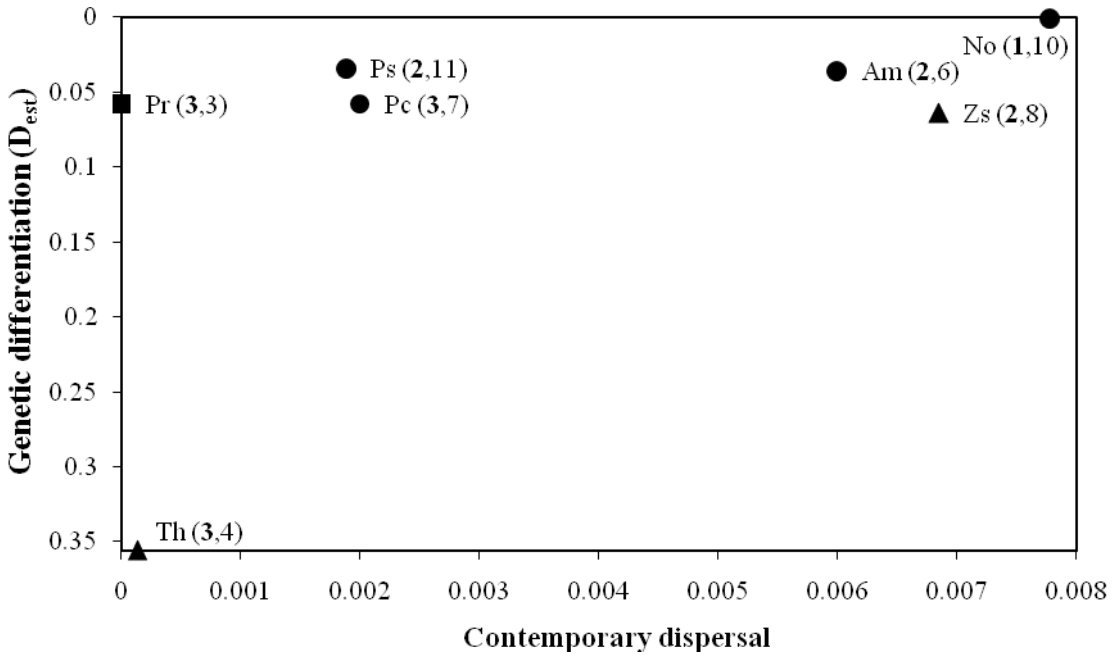

Figure 1.2. Genetic population differentiation versus contemporary dispersal in seven bird species of the Taita Hills (SE Kenya). Species abbreviations are No, *Nectarinia olivacea;* Ps, *Pogonocichla stellata;* Zs, *Zosterops (poliogastrus) silvanus;* Am, *Andropadus milanjensis;* Pc, *Phyllastrephus cabanisi;* Pr, *Phylloscopus ruficapilla;* Th, *Turdus helleri.* High D_{est} values correspond to low levels of historic gene flow. Values in brackets indicate numbers of genetic clusters (bold font) and forest patches occupied (regular font); ● no mutation/drift or migration/drift disequilibrium; ▲ mutation/drift disequilibrium only; ■ mutation/drift and migration/drift disequilibrium. Contemporary dispersal rates (ψ–values corrected for species-specific recapture/survival probabilities and inter-fragment distances) and patch occupancy data from Lens *et al.* (2002).

DISCUSSION

Loss of gene flow due to reduced dispersal, and reduction in effective population size due to genetic drift, can re-distribute genetic variability among spatially-structured populations over the course of a few generations only (Harrison & Hastings 1996). Within the isolated Taita Hills of south-east Kenya, severe fragmentation of the original indigenous forest cover resulted in varying levels of population subdivision among seven sympatric, ecologically related forest bird species. There was strong consensus among species in the geographic patterning of genetic variation, reflecting the important role of landscape structure in genetic clustering and population differentiation (Storfer *et al.* 2007). For instance, in all three species that comprise two genetic clusters, cluster MB was consistently separated from cluster NG-CH and between-cluster genetic differentiation was always higher than within-cluster differentiation. The strong isolation of populations in fragment MB from those in fragments CH and NG is likely due to the presence of a low-altitude valley that reduced gene flow levels even prior to human-induced deforestation (Brooks *et al.* 1998, Pellikka *et al.* 2009). When combining information on past and current mobility, however, the level of correspondence between both sources of information was only weak. While two species clearly stood out as having either low or high mobility according to both historic and current estimates (*T. helleri* and *N. olivacea*, respectively), five other species showed highly variable current dispersal rates despite broadly similar levels of genetic population differentiation.

The most contrasting species pair in terms of current and historic mobility were *P. ruficapilla* and *T. helleri*, both highly sedentary species with (near) zero current dispersal but relatively low and very high levels of genetic differentiation, respectively. While genetic differentiation, reflecting gene flow over many generations, cannot be compared in absolute terms with dispersal events that may not result in reproduction (Slatkin 1987; Koenig *et al.* 1996; Thompson & Goodman 1997; Whitlock & McCauley 1999; Hedrick 2005a; Holsinger & Weir 2009; Lowe & Allendorf 2010), contrasting both estimates suggests that *P. ruficapilla* suffered a severe relative loss of mobility over time. While both species showed evidence of a genetic bottleneck in one or more populations, *P. ruficapilla* was the only species that also showed evidence of migration/drift disequilibrium, hence confirming our interpretation of a recent decrease in gene flow among its remnant populations. Lack of evidence for a similar decrease in gene flow in *T. helleri*, suggests that the extremely low mobility of this critically-endangered species is not of recent origin. Earlier, radio-telemetric data showed that

individuals forage and move strictly within indigenous forest boundaries, with no evidence of excursions into the landscape matrix (Lehouck *et al.* 2009). Such a strong dependence of *T. helleri* on prime indigenous forest matches with its exceptionally high level of stress sensitivity inferred from spatiotemporal patterns in tarsus asymmetry, a presumed proxy of environmental stress (Lens *et al*. 1999). Stress-sensitive forest specialists such as *T. helleri* can be expected to have become locked in very early in the fragmentation process, which is thought to have been ongoing for several hundred years in the Taita Hills (Pellikka *et al.* 2009). In contrast, more generalist low-mobility species, such as *P. ruficapilla*, may have been able to persist longer in degraded forest patches, allowing gene flow to continue for a longer time during the fragmentation process. However, progressing deterioration of these small stepping-stone patches (an estimated 50% of indigenous forest cover was lost to agriculture and admixture with secondary growth and exotic plantations between 1955 and 2004; Pellikka *et al.* 2009) may have resulted in a strong and recent reduction in mobility, explaining the migration/drift disequilibrium of *P. ruficapilla* with evidence for a recent bottleneck in fragment MB, the largest, most pristine, but also most isolated fragment of the Taita archipelago.

Two other species with intermediate levels of genetic differentiation, *P. stellata* and *P. cabanisi*, showed low to moderate dispersal rates, suggesting moderate losses in mobility over time, however without evidence for migration/drift disequilibrium. While individuals of *P. cabanisi* were assigned to three genetic clusters, *P. stellata* showed only two clusters. Such a difference in genetic clustering among two species with comparable contemporary dispersal rates may result from variation in sensitivity to habitat disturbance. Unlike all other study species, *P. cabanisi* forages and breeds in small family groups (T. Callens, *unpubl. data*). Group-living species are thought to exceed the carrying capacity of small and disturbed habitat remnants more easily and pay higher costs of biotic interactions near habitat edges and in the landscape matrix than pair-living species (Van Houtan *et al.* 2006). This may explain why breeding populations of *P. cabanisi* are currently absent from nearly half of the Taita forest remnants (Lens *et al.* 2002). In contrast, *P. stellata* breeds in most of the small forest patches, including highly degraded forest remnants as small as 0.2 ha (Spanhove *et al.* 2009). Given such tolerance to degraded habitat, *P. stellata* dispersers from large, intact forest fragments may regularly settle in small, degraded remnants scattered across the landscape, resulting in step-wise gene flow between the larger fragments. Alternatively, discrepancies between historic and current estimates of mobility might result from a downward bias in

dispersal estimates, e.g. because fledglings are only captured post-dispersal (see Van Treuren *et al.* 1999). In *P. cabanisi*, 127 nestlings (ringed 2007-2009) yielded 26 retraps, none of which originated from another fragment; while in *P. stellata*, 282 nestlings (ringed 2002-2005) yielded 50 retraps, 5 of which originated from another fragment (T. Callens & T. Spanhove, *unpubl. data*). While these limited datasets prevent us from formally testing variation in timing of natal dispersal between both species, they do suggest that the accuracy of dispersal rates estimated from post-fledgling mark-recapture data may differ among species (see Desante 1995; Thomson *et al.* 1999). Yet, given that dispersal estimates used to infer current mobility rates in this paper were statistically corrected for between-species heterogeneity in survival and recapture rates and did not comprise recapture events of ringed nestlings (Lens *et al.* 2002), they are still considered valid for comparison of relative mobility rates.

Results from this study hence confirm the conclusion from other multi-species studies that sympatric forest birds may differ strongly in genetic signature of forest fragmentation (Bates 2002; Brown *et al.* 2004; Burney & Brumfield 2009). More importantly, our study shows that such differences may also appear among species with broadly similar levels of current mobility. In line with this, Van Houtan *et al.* (2006, 2007) hypothesized that post-fragmentation levels of vagility may not be the best predictor of how forest fragmentation affects the life history of forest-dependent species. Rather, species which range more widely pre-fragmentation, e.g. those that track unpredictable food resources or frequently join (mixed-species) flocks, may be more vulnerable to post-fragmentation extinction compared to species that do not cross gaps as often. Earlier, we proposed that conservation tactics in the fragmented Taita ecosystem may fail unless they include action both within sites, to minimize habitat deterioration, and across sites within the landscape, to maximize dispersal (Lens *et al.* 2002). As part of a series of conservation initiatives funded by Conservation International, over 150000 indigenous tree seedlings have been raised in community-owned tree nurseries since 2006. To address within-site issues, initial efforts are being undertaken to restore disturbed sections within the indigenous forest fragments. For between-site action, priority areas for reforestation within the matrix were selected based on a combination of least-cost modeling analysis (where we quantified landscape connectivity and identified likely dispersal corridors linking the indigenous fragments; Adriaensen *et al.* 2006), forest ecology (silvicultural) characteristics and sociological aspects (Githiru & Lens 2007). Findings from the current study provide evidence that habitat isolation results in reduced genetic

connectivity, but most importantly, show that not all species are equally sensitive. Combining landscape, demographic and behavioral data with population genetic data, such as presented here, therefore helps to determine which species may be most prone to extinction, and hence, to rationalize conservation action at species level (Pavlacky *et al.* 2009; Lowe & Allendorf 2010). For instance, while conservation action for *P. ruficapilla* should primarily focus on the restoration of landscape connectivity through creation of small (stepping-stone) forest patches, such action may not suffice for *T. helleri* unless combined with habitat restoration programs in all occupied forest fragments. For *P. stellata* and *P. cabanisi*, the apparent loss in current versus historic mobility and different patterns of genetic clustering merely act as an early warning system against future losses in gene flow and genetic variation.

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CONTRIBUTIONS

Blood and feather samples for this chapter were obtained from previous research activities (Taita Hills Biodiversity Project). J.R. Huyghe and V. Vandomme executed the major part of the DNA extraction and genotyping work. T. Callens analyzed and interpreted all genotyped data and wrote the article. E.Y. Durand assisted with the genetic clustering analyses in TESS. P. Galbusera, L. Lens and E. Matthysen helped to establish the conceptual framework of the paper, and together with M. Githiru, they all assisted throughout the writing phase of this manuscript, greatly improving the quality of it.

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APPENDICES

Appendix 1.A. Microsatellite DNA specifications and PCR conditions for seven bird species of the Taita Hills, SE Kenya.

Locus	Species[1]	Reference	Product size (bp)	Hybridisation temp. (°C)	Concentration $MgCl_2$ (mM)
Nectarinia olivacea					
Pc3	*Parus caeruleus*	Dawson *et al.* 2000	141-155	50	1.0
Pocc8	*Phylloscopus occipitalis*	Bensch *et al.* 1997	220-222	55	2.0
Ppi2	*Pica pica*	Martinez *et al.* 1999	229-243	55	2.5
Gf6	*Geospiza fortis*	Petren 1998	166-180	56	2.5
Pc9	*Parus caeruleus*	Dawson *et al.* 2000	143-165	55	1.5
Pat14	*Parus atricapillus*	Otter *et al.* 1998	126-130	50	1.0
Ls1	*Lanius ludovicianus*	Mundy & Woodruff 1996	190-214	50	1.5
Pdo1	*Passer domesticus*	Neumann & Wetton 1996	162-170	50	2.0
Pogonocichla stellata					
Pat14	*Parus atricapillus*	Otter *et al.* 1998	143-173	50	1.5
Mcyμ4	*Malurus cyaneus*	Double *et al.* 1997	132-152	55	1.0
Ltmr6	*Chiroxiphia linearis*	McDonald & Potts 1994	190-198	54	2.0
Gf6	*Geospiza fortis*	Petren 1998	138-154	56	2.5
WBSW2	*Plocepasser mahali*	McRae & Amos 1999	125-131	54	1.5
WBSW9	*Plocepasser mahali*	McRae & Amos 1999	102-122	54	1.5
GF5B	*Geospiza fortis*	Petren 1998	199-227	57	1.5
Zosterops silvanus					
Mcyμ4	*Malurus cyaneus*	Double *et al.* 1997	137-161	55	2.5
Cu28	*Catharus ustulatus*	Gibbs *et al.* 1999	166-168	60-51	2.5
Zl12	*Zosterops lateralis*	Degnan *et al.* 1999	110-120	57	1.5
Pocc1	*Phylloscopus occipitalis*	Bensch *et al.* 1997	222-246	55	2.0
Andropadus milanjensis					
Pdo1	*Passer domesticus*	Neumann & Wetton 1996	164-168	50	2.0
WBSW2	*Plocepasser mahali*	McRae & Amos 1999	226-234	45	1.0
Pc3	*Parus caeruleus*	Dawson *et al.* 2000	165-171	50	1.0
Mcyμ4	*Malurus cyaneus*	Double *et al.* 1997	134-138	55	2.5
WBSW11	*Plocepasser mahali*	McRae & Amos 1999	162-176	46	1.0
Pat14	*Parus atricapillus*	Otter *et al.* 1998	136-138	50	1.0
Dpu16	*Dendroica petechia*	Dawson *et al.* 1997	151-157	52	1.0

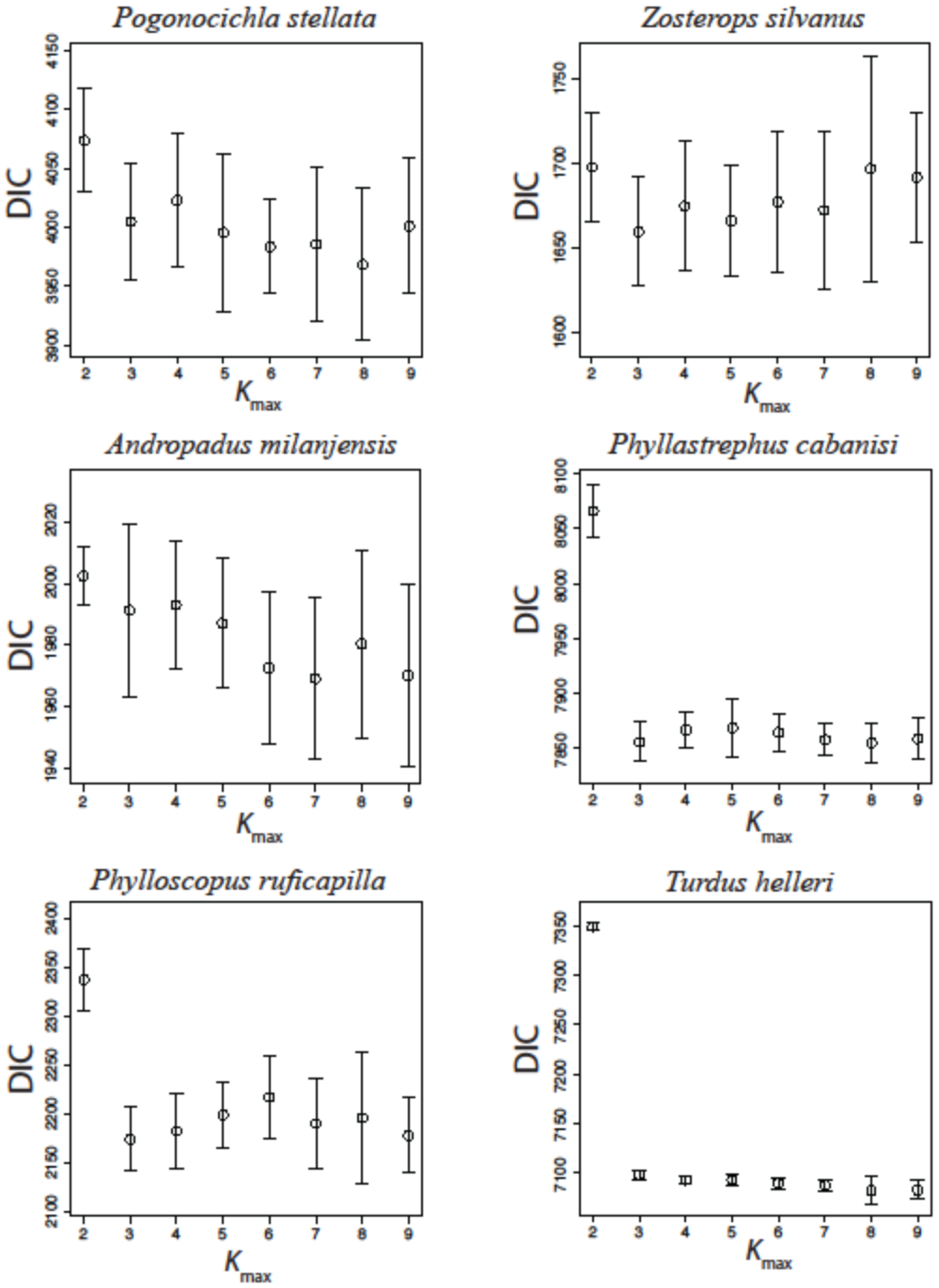
[1]Species for which the primer was originally developed.

Appendix 1.A. continued

Locus	Species[1]	Reference	Product size (bp)	Hybridisation temp. (°C)	Concentration $MgCl_2$ (mM)
Phyllastrephus cabanisi					
Ase18	*Acrocephalus sechellensis*	Richardson *et al.* 2000	236-353	57	2.0
Indigo41	*Vidua chalybeata*	Sefc *et al.* 2001	276-312	57	2.0
Ls1	*Lanius ludovicianus*	Mundy & Woodruff 1996	164-220	50	1.5
Ls2	*Lanius ludovicianus*	Mundy & Woodruff 1996	191-200	50	1.5
Mcyμ4	*Malurus cyaneus*	Double *et al.* 1997	131-159	55	1.5
Pc3	*Parus caeruleus*	Dawson *et al.* 2000	157-169	53	1.0
Pc4	*Parus caeruleus*	Dawson *et al.* 2000	152-164	53	2.0
Pfi04	*Phyllastrephus cabanisi*	R.C.K. Bowie, unpublished data	139-201	57	1.5
Pfl54	*Phyllastrephus cabanisi*	R.C.K. Bowie, unpublished data	222-251	57	2.0
WBSW2	*Plocepasser mahali*	McRae & Amos 1999	212-230	45	1.0
Phylloscopus ruficapilla					
Cu02	*Catharus ustulatus*	Gibbs *et al.* 1999	154-162	60-48	2.5
Pat43	*Parus atricapillus*	Otter *et al.* 1998	117-125	55	1.0
Zl18	*Zosterops lateralis*	Degnan *et al.* 1999	177-191	55	1.5
Mslp4	*Locustella pryeri*	Ishibashi *et al.* 2000	136-140	55	1.5
Pocc1	*Phylloscopus occipitalis*	Bensch *et al.* 1997	229-233	52	1.0
Pocc8	*Phylloscopus occipitalis*	Bensch *et al.* 1997	214-218	55	2.0
Dpu16	*Dendroica petechia*	Dawson *et al.* 1997	152-154	50	1.5
Turdus helleri					
Ltmr6	*Chiroxiphia linearis*	McDonald & Potts 1994	214-226	55	2.0
Pc3	*Parus caeruleus*	Dawson *et al.* 2000	115-125	52	2.0
Pat 43	*Parus atricapillus*	Otter *et al.* 1998	141-171	54	1.5
GF5B	*Geospiza fortis*	Petren 1998	199-227	57	1.5
Mjg1Te	*Aphelocoma ultramarina*	Li *et al.* 1997	100-172	55	1.5
Pdo5	*Passer domesticus*	Griffith *et al.* 1999	266-274	57	2.5

[1]Species for which the primer was originally developed.

Appendix 1.B. Bayesian admixture model selection in TESS 2.3. For each study species, DIC values are plotted against K-values (maximal number of clusters). Values corresponding to DIC values that first level off, are selected.



Appendix 1.C. Cluster-level excess of heterozygote genotypes in seven bird species of the Taita Hills (SE Kenya) with CH = Chawia, MB = Mbololo and NG = Ngangao. Wilcoxon-based levels of significance are shown for different mutation models (all $P > 0.05$).

Species	Population Cluster	TPM[1]	SMM[2]
Nectarinia olivacea	CH - MB - NG	0.770	0.809
Pogonocichla stellata	CH - NG	0.656	0.980
	MB	0.992	1.000
Zosterops silvanus	CH - NG	0.563	0.906
	MB	0.156	0.156
Andropadus milanjensis	CH - NG	0.656	0.766
	MB	0.531	0.656

[1]Two-Phase Model; [2]Stepwise Mutation Model

Fieldwork in the Taita Hills. © Tom Callens

Post-fragmentation dynamics in an Afrotropical cloud forest bird: a spatiotemporal approach

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ABSTRACT

How demography and genetics interact in their effect on population viability is a long-standing issue in the study of fragmented populations. Census and effective population sizes and gene flow constitute key parameters to quantify such effects, and demographic and genetic tools are currently available to estimate these properties with increasing precision. Here we analyze demographic and genetic changes over a recent fifteen year time span in five remnant populations of a group-living bird, cabanis's greenbul (*Phyllastrephus cabanisi*), that experienced moderate historical loss of mobility following severe fragmentation of its cloud forest habitat. Contrary to our expectation, genetic variation, genetic admixture, and effective population sizes increased from a first (1996-2000) to a second (2006-2010) time period. In spite of small effective population sizes in tiny forest remnants, none of the populations showed evidence for a recent population bottleneck. Heterogeneity in the ratio of effective to census population sizes among populations suggests that the mechanisms underlying temporal shifts in genetic population parameters varied, likely reflecting differential shifts in immigration versus emigration rates. Generically, results from this study appeal for the integration of demographic, genetic, ecological and behavioral data when assessing long-term population dynamics in fragmented landscapes.

INTRODUCTION

How genetics and demography interact in their effects on population viability is a long-standing issue in conservation biology (Richards *et al.* 2003). Currently, there is a growing consensus that as populations become smaller and fragmented into increasingly smaller habitat patches, their viability is determined by both demographic and genetic stochastic processes, and possibly by their interaction (Lande 1988, Ellstrand & Elam 1993, Frankham 1995, Young & Clarke 2000, Amos & Balmford 2001). The effective size of a population (N_e, Wright 1931) estimates the rate at which genetic variance changes due to stochastic genetic drift (Crow & Kimura 1970) and constitutes a key parameter in the genetic study of small populations. Natural populations generally do not adhere to idealized settings (Whitlock & McCauley 1999) and demographic processes such as fluctuations in population size (Frankham 1995, Vucetich *et al.* 1997), unequal sex ratios (Frankham 1995) or large variance in reproductive success due to high fecundity or polygamy (Nunney 1996, Storz *et al.* 2001) may reduce N_e relative to the absolute number of individuals alive (census population size, N_c) (Caballero 1994, Leberg 2005). Based on theoretical expectations and empirical estimates, ratios of effective to census population sizes (N_e/N_c) have been shown to range between 0.1-0.5 in natural populations of birds (Nunney 1993, Frankham 1995, Brown *et al.* 2007, Palstra & Ruzzante 2008). However, some studies revealed substantial variation within single species (Ardren & Kapuscinski 2003, Watts *et al.* 2007, Palstra & Ruzzante 2008).

Studies of N_e/N_c have proven particularly informative when evaluating population effects of habitat change, such as fragmentation of formerly continuous habitat blocks into small, isolated remnants. Habitat fragmentation can affect the genetic population structure of species both directly and indirectly, i.e. by restricting gene flow, reducing population sizes, or increasing genetic drift and inbreeding in small relict populations (reviewed by Frankham *et al.* 2002). While low N_e/N_c ratios may imply that such fragmented populations become more sensitive to genetic stochasticity at levels where census populations sizes would not provide such indication (Palstra & Ruzzante 2008), high ratios may be indicative for genetic compensation through changes in biological interactions that reduce the rate of genetic erosion, as has been reported for small populations of invertebrates, fishes and frogs (Ardren & Kapuscinski 2003, Watts *et al.* 2007, Ficetola *et al.* 2009). When genetic erosion is correlated with a reduction in lifetime fitness components, positive feedbacks between population size and genetic diversity may ultimately increase the probability of population

extinction (Richards *et al.* 2003, Johnson *et al.* 2004). However, new input of individuals through dispersal may dampen demographic and genetic effects of population fragmentation by increasing population growth rates or genetic variation (Hanski & Gilpin 1991, Clobert *et al.* 2001, Waples 2010).

Effective population sizes and gene flow hence constitute key genetic parameters to quantify population effects of habitat fragmentation, and different methods have been developed to estimate these parameters in natural populations based on life history data, lethal alleles or linkage disequilibrium between genetic loci (reviewed by Caballero 1994, Leberg 2005, Wang 2005). While traditional estimates of N_e invariably assumed single isolated populations that received no immigrants over the study interval, dispersal and gene flow are generally not negligible and can substantially bias estimates of N_e if not properly accounted for (Wang & Whitlock 2003). More recently, genetic methods have been developed based on the premise that temporal variance in neutral genetic allele frequencies is inversely proportional to the effective population size. While originally developed for species with discrete generations, these methods have subsequently been extended to cases where generations overlap and, most importantly, for which the assumption of strict population isolation is not met (Luikart *et al.* 2010 and references therein).

Here we use a spatiotemporal approach to study changes in census and effective population sizes within, and genetic admixture and gene flow among, fragmented populations of a forest-restricted bird species in an African rainforest archipelago, by integrating spatial and temporal demographic and genetic data collected over 15 years. Cabanis's greenbul (*Phyllastrephus cabanisi*) lives in small family groups year-round and we earlier showed indications of moderate loss of mobility over time following extreme fragmentation of its rainforest habitat ca. 50 years ago (Callens *et al.* 2011). Because group-living species are thought to exceed the carrying capacity of small and disturbed habitat remnants more easily and to pay higher costs of biotic interactions near habitat edges and in the landscape matrix than pair-living species (Van Houtan *et al.* 2006), low post-fragmentation levels of N_e and gene flow might render this species vulnerable to local extinction. To study if, and to what extent, contemporary populations of *P. cabanisi* show evidence of ongoing genetic erosion and isolation, we first compare genetic variation, effective and census population sizes, and genetic population structure between two non-overlapping time periods (1996-2000 and 2006-2010). Next, we assess the relative effect of gene flow on estimates of effective population size by jointly

estimating N_e and m from temporal changes in allele frequencies across both time periods, with and without taking gene flow into account (Wang & Whitlock 2003, Fraser *et al.* 2007a). Spatiotemporal patterns in N_e, N_c, genetic admixture and gene flow are related to ecological and behavioral properties of *P. cabanisi* and interpreted within a metapopulation framework.

MATERIAL AND METHODS

Study area and species

The Taita Hills (SE Kenya, 03°24'S, 38°21'E) represent the northernmost extreme of the Eastern Arc Mountains, a chain of mountains that run from south-eastern Kenya to southern Tanzania (Lovett & Wasser 1993) and boast a high diversity of flora and fauna, high levels of endemism, and high levels of threat (Mittermeier *et al.* 1998, Newmark 1998, Stattersfield *et al.* 1998). Indigenous forests of the Eastern Arc are currently scattered over ca. 5076 km² (Platts *et al.* 2010) and are strongly isolated from other East-African mountain forests. Forest loss within the Eastern Arc Mountains has been both rapid and drastic. Within the Taita section, the total area of indigenous forest cover decreased by ca. 50% between 1955 and 2004 (Pellikka *et al.* 2009), mainly due to clearance for small-subsistence agriculture (Bennun & Njoroge 1999, Myers *et al.* 2000). At present, the Taita forest archipelago covers 430 ha of indigenous forest fragmented into three larger patches (between 86 and 185 ha) and eight tiny remnants (2-8 ha) that are located on two mountain isolates (Dabida and Mbololo) separated by a low-altitude valley (Paranga) (Lovett 1985, Pellikka *et al.* 2009) (Fig. 2 General Introduction). Changes in the total area of indigenous tree cover in forest fragments that held *P. cabanisi* breeding populations were based on visual analysis of temporal series of black-and-white aerial photography (1993) and airborne digital camera mosaics (2004) (Pellikka *et al.* 2009). After correction for spectral distortions following Pellikka (1998), 1993 photos were georeferenced to mosaics using Erdas Imagine while 2004 photos were mosaicked and orthorectified using EnsoMOSAIC (Holm *et al.* 1999). Resulting land cover data were transferred to a Transverse Mercator projection with a Clarke 1880 spheroid and Arc 1960 datum resampled to 0.5 m ground resolution. Changes in forest cover (geometric accuracy within 2 m as ground truthed with GPS readings) were analyzed with ArcGIS 10 (Pellikka *et al.* 2009).

P. cabanisi is a medium-sized passerine that inhabits central- to east-African moist forest (Keith *et al.* 1992, Bennun *et al.* 1996). Globally, the IUCN conservation status of the species is currently categorized as 'Least Concern' (Birdlife International 2012). Within the Taita archipelago, the species has been recorded in eleven indigenous forest fragments though breeding is restricted to the largest three (MB 185 ha, NG 120 ha, CH 86 ha) and two small (FU 8 ha, ND 4 ha) fragments (Fig. 2 General Introduction: NG, CH, FU and ND located on a single mountain isolate). In one other tiny remnant Macha (MA 2.5 ha), individuals have unsuccessfully attempted to breed since 2007. *P. cabanisi* is a facultative cooperative breeder that lives in small family groups (breeding pair and 0-3 helpers, Chapter 4), occasionally feeds on fruits but supplies nestlings with invertebrates and small vertebrates (Keith *et al.* 1992, Lehouck *et al.* 2009). Compared to six sympatric forest species, *P. cabanisi* appears moderately sensitive to indigenous forest disturbance (Lens *et al.* 1999), while a comparison of past population differentiation (estimated from microsatellite genotypes) with contemporary dispersal rates (estimated from capture-recapture data) indicated moderate loss of mobility over the last five decades (Callens *et al.* 2011). Between 1996 and 2010, a total of 2285 birds were trapped, aged (following Jackson 2005), marked, measured, sampled and released at the original site of capture in fragments MB, NG, CH, FU and ND. Mist-net lines were operated in one to seven 4-ha plots per fragment (depending on fragment size) and were evenly spaced out in order to sample entire plots. Net positions, net lengths (120m/plot) and daily trapping efforts (06-18h) were kept constant between trapping sessions. Time intervals between subsequent ringing sessions varied between 1.0 and 4.6 months, and the number of ringing sessions per fragment ranged between 20 and 32 over the 15 year study period.

Genetic variation

Upon first capture of an individual, 2-3 µl of blood was collected from the brachial vein and stored in a 95% ethanol buffer. DNA was isolated by boiling in a 5% Chelex solution (Biorad) after an incubation period of 90 min at 55°C in the presence of 100 µg proteinase K (Walsh *et al.* 1991). Polymerase chain reactions (PCR) were organized in three multiplex sets and performed in a final volume of 5 µl containing $\leq$ 20ng DNA/µl and an 1:1 ratio of the QIAGEN® Multiplex PCR Master Mix (providing a final concentration of 3 mM MgCl2) to primermix (multiplex sets and concentrations in Appendix 2.A). The applied PCR profile included an initial HotStart Taq activation step of 15 min at 95°C, followed by 35 cycli of 30 s at 94°C, 90 s at 57°C and 60 s at 72°C, followed by an additional elongation step of 30 min at 60°C. PCR products were visualized on an ABI3130 sequencer (Applied Biosystems) and

microsatellite lengths were determined with GENEMAPPER 4.1. Two random subsets of genotypes were drawn that corresponded to individuals captured during one of two equal time periods: first period (1996-2000): MB = 62 ind, NG = 65 ind, CH = 65 ind, FU = 14 ind, ND = 18 ind ; second period (2006-2010): MB = 54 ind; NG = 54 ind; CH = 55 ind; FU = 24 ind; ND = 16 ind. Temporal genetic samples were hence separated by a period of six years, which we assumed to correspond to one *P. cabanisi* generation. The age structure of sampled individuals in both periods did not differ, and individuals sampled during the first period and still alive during the second one, were included in the first period only.

We used MICROCHECKER 2.2.3 (Van Oosterhout *et al.* 2006) to identify scoring errors that could be attributed to stuttering, differential amplification of size-variant alleles causing large allele drop-out or presence of null alleles. Running 10000 Monte Carlo simulations and calculating 95% confidence did not provide evidence for null alleles in any of the loci under study. Deviations from Hardy–Weinberg equilibrium and linkage disequilibrium per locus were tested with GENEPOP 4.0 (Raymond & Rousset 1995, Rousset 2008). All loci were consistent with Hardy-Weinberg equilibrium (all $P > 0.05$ after Bonferroni correction, Rice 1989) and linkage disequilibrium was only significant between Pca3-WBSW2 (period 1) and Pfi04-Pfl54 (period 2) after correction for multiple testing (Rice 1989). Allelic richness corrected for sample size (see Leberg 2002) was calculated with FSTAT 2.9.3.2 (Goudet 1995), while observed and expected levels of heterozygosity were calculated with GENALEX 6.0 (Peakall & Smouse 2006).

Deviation from mutation-drift equilibrium was tested by comparing levels of heterozygosity calculated from observed allele frequencies (*sensu* Nei *et al.* 1975, Nei 1987) with those expected under equilibrium with BOTTLENECK 1.2.02 (Piry *et al.* 1999). Because the mutation model underlying the microsatellite markers was unknown, data were analyzed both under the two-phase (TPM) and stepwise mutation model (SMM) (Luikart & Cornuet 1998, Di Rienzo *et al.* 1994, Jarne & Lagoda 1996, Piry *et al.* 1999). When modeling TPM models, combinations of 95% single-step mutations and 5% multistep mutations were used, with a variance of 30 among multiple-step mutations (10^4 replications) (Piry *et al.* 1999). Deviation from migration-drift equilibrium was tested by comparing the relative likelihoods of 'gene flow-drift' and 'drift only' models with 2MOD 0.2, using a MCMC procedure with 10^5 iterations and a burn-in of 10^4 (Ciofi *et al.* 1999). Time intervals between population founding and population sampling were assumed sufficiently short such that effects of mutations were

negligible (drift only), while mutation rates were assumed much smaller than gene flow rates (gene flow-drift). Data were analyzed under different mutation models and Bayes factors were calculated to infer decisive power estimates of the most likely models (Jeffreys 1961, Goodman 1999).

Census and effective population sizes

We derived encounter histories from capture-recapture data for all individuals trapped during the first (N = 364 ind) or second (N = 661 ind) period. Next, we applied POPAN models (Schwarz & Arnason 1996) implemented in program MARK 6.0 (White & Burnham 1999) to estimate population- and period-specific recapture rates (*p*), yearly apparent survival rates (Φ), probabilities of entry into the population per session (*pent*), and total numbers of individuals present (*N*). We evaluated time dependent (~*t*), linear time trend (~*T*), and constant (~1) formulations for Φ, *p* and *pent* and additionally evaluated effects of sampling effort by introducing a linear dependence on the number of sampling days during each session. Models were run for all alternative combinations of parameter formulations and were ranked according to Akaike's Information Criterion (AICc) values corrected for small sample size (Hurvich & Tsai 1991). To account for model uncertainty, we derived model-averaged estimates of *N* averaged over all models based on AICc weights and extrapolated the harmonic mean of each period to the total area of indigenous forest (N_c). Standard errors were extrapolated using the delta method and transformed into 95% CI-intervals.

To estimate effective population sizes (N_e), two different approaches were used: a single-sample estimator and a temporal two-sample estimator. First, we used LDNe 1.31 (Waples & Do 2008) to calculate single-sample estimates based on linkage disequilibrium (N_{eLD}). Principally, this method builds upon the fact that as N_e decreases, non-random association of alleles at different loci will occur, resulting in gametic disequilibrium or linkage disequilibrium (Hill 1981, Waples 1991). Recently, this method has been corrected for biases due to small sample sizes (Waples 2006, Waples & Do 2008) and it performs well under skewed sex ratios or non-random variance in reproductive success (Waples 2006, England *et al.* 2006). To reduce estimation bias due to the effect of rare alleles (Waples & Do 2008, 2010), a random mating model and critical allele frequency value of 0.02 were applied. We ran the program on samples of the first and second period, with the calculated estimates reflecting effective population sizes one generation prior to the moment of sampling (see details in Waples 2005). Therefore, given the fact that both periods are separated by one

generation, the N_{eLD} estimates calculated on samples from the second period, basically apply to the first period (Table 2.1). Differences in N_{eLD} estimates calculated in both periods were tested with Wilcoxon signed rank tests (StatXact 5.0.3).

Second, we used MLNE 2.3 (Wang & Whitlock 2003) to estimate variance effective population sizes (N_{eV}) based on samples taken at two different time points (first and second period). This temporal method estimates the harmonic mean of N_e over the period between the samples on the basis of changes in allele frequencies produced by genetic drift. As not only genetic drift, but also migration rates can change allele frequencies over time, the advantage of this program is that it can simultaneously estimate effective population sizes (N_{eV}) and migration rates (m), resulting in less biased values of N_{eV} compared to programs that assume isolated populations (Wang & Whitlock 2003). To quantify putative effects of gene flow on estimates of N_{eV}, effective population sizes were estimated *with* (open models) and *without* (closed models) taking into account gene flow between populations (i.e. from all non-focal populations pooled or from each of the large populations separately) (Wang & Whitlock 2003, Fraser *et al.* 2007a). After setting the maximum N_{eV} value to 10000, pseudo-maximum-likelihood estimates of N_{eV} (with 95% CI) were computed assuming a single generation interval between both time periods. Therefore, the calculated N_{eV} estimates based on allele frequency shifts from a first to a second period, are essentially effective population size estimates for the first period (see details in Waples 2005). Finally, the N_{eV} estimates from the open models were divided by the harmonic mean estimates of N_c to calculate N_e/N_c ratios from the first period in each population (Table 2.2).

Genetic population structure

Genetic population structure during the first and second period was inferred from spatially-explicit Bayesian admixture models implemented in TESS 2.3 (Chen *et al.* 2007, Durand *et al.* 2009). Spatially explicit methods are more powerful than non-spatial algorithms at low F_{ST} values (Chen *et al.* 2007, Durand *et al.* 2009) and more robust to infer population structure resulting from fission (fragmentation) and fusion (gene flow) events (François & Durand 2010). The number of genetic clusters (K) was restricted between 1-9, and a total of 100 independent iterations (each 50000 sweeps long and discarding the first 25000 sweeps) were run for each value of K. Model fits were compared with the Deviance Information Criterion (DIC, Spiegelhalter *et al.* 2002), a penalized measure of fit accounting for model complexity (models with lower DIC values fitting the data better). DIC values averaged over 100

independent iterations were plotted against K, and K-values for which DIC values first reached a plateau, were selected (procedure similar to the 'logarithm of evidence' one in STRUCTURE, Evanno *et al.* 2005). Ten runs with lowest DIC values for the selected K-value were retained, and their admixture estimates were averaged using CLUMPP 1.1.1 (Jakobsson & Rosenberg 2007), applying the greedy algorithm with random input order and 1000 permutations to align the runs and calculate G' statistics. Results were visualized using DISTRUCT 1.1 (Rosenberg 2004). To assess whether the level of population admixture differed between periods, we first built a matrix Q_F per period with element (i,j) equaling 1 if individual *i* was sampled in fragment *j*, and 0 otherwise. We then computed the correlation coefficient between Q_F and the estimated admixture coefficients for each period. 95% confidence intervals for the two correlation coefficients were computed after Fisher Z-transformation.

RESULTS

Genetic variation

Levels of allelic richness increased from the first to the second period in all populations except NG (Table 2.1). Observed and expected levels of heterozygosity based on Hardy-Weinberg equilibrium did not differ among populations or periods. None of the populations in either period showed evidence of heterozygosity excess compared to levels expected under mutation-drift equilibrium (Wilcoxon's signed rank test; TPM and SMM: all $P > 0.05$). However, during the second period, populations NG and FU showed heterozygosity deficiency compared to levels expected under mutation-drift equilibrium (Wilcoxon's signed rank tests; NG: TPM: $P = 0.082$ (almost significant); SMM: $P = 0.024$; FU: TPM: $P = 0.003$; SMM: $P = 0.001$).

Population sizes and survival rates

Estimates of N_c ranged between 334-995 in the three large populations, and between 10-13 in the two small ones (Table 2.1). During the second period, N_c estimates were significantly larger in NG (+42%; $t_{241} = -4.21$; $P < 0.0001$) and lower in CH (-21%; $t_{222} = 3.56$; $P = 0.0006$) while trends in the other populations were less clear-cut due to overlapping 95% CI's (Table 2.1). Overall, estimates of N_{eLD} tended to increase during the second sampling period (Wilcoxon's signed rank test; $Vs = 15$; $P = 0.06$), most strongly in populations ND and CH

(Table 2.1). When pooling all non-focal populations as putative source of migrants, N_{eLD} estimates (second period, but applying to the first period, see material and methods) corresponded to N_{eV} estimates (applying to the first period, see material and methods), although the latter tended to yield higher estimates in NG and FU (Table 2.2). Under presumed absence of dispersal (*closed* models), N_{eV} estimates were consistently higher (with infinite confidence intervals) for all populations except NG (Table 2.2). N_e/N_c ratios varied between 0.08 and 0.26 among large fragments, while ratios in both small populations were markedly larger and even higher than 1 (Table 2.2). Mean yearly survival rates did not differ between periods (Wilcoxon's signed rank test; Vs = 8; P = 0.89), while 95% CI intervals largely overlapped among fragments (Table 2.1).

Table 2.1. Estimates of allelic richness corrected for sample size (A_R), observed (H_O) and expected heterozygosity (H_E), yearly apparent survival (Φ), effective population sizes (N_{eLD}) and census population sizes (N_c) in five *P. cabanisi* populations sampled during two non-overlapping periods (Period 1: 1996-2000; Period 2: 2006-2010). N_{eLD} estimates (with 95 % confidence intervals obtained through jackknifing across loci) were obtained from a linkage disequilibrium model implemented in LDNe 1.31. N_c and Φ estimates (with 95 % confidence intervals) were obtained from POPAN models implemented in MARK 6.0 and extrapolated to the total area of indigenous tree cover prior to the first (1993, upper panel) and second (2004, lower panel) period (vegetation data obtained from Pellikka *et al.* 2009 and complemented with unpublished data).

Population	Indigenous tree cover (ha)	A_R	H_O	H_E	Φ (CI)	N_{eLD} (CI)	N_c (CI)
Period 1							
CH	93.4	4.9	0.631	0.603	0.75 (0.71-0.80)	55 (38-88)	424 (332-516)
FU	8.6	4.5	0.557	0.573	0.69 (0.58-0.80)	8 (4-13)	12 (8-16)
MB	185.2	4.5	0.615	0.614	0.74 (0.66-0.82)	67 (40-144)	845 (641-1049)
ND	4.0	3.8	0.567	0.532	0.75 (0.63-0.87)	4 (3-7)	13 (7-19)
NG	118.8	5.2	0.605	0.583	0.72 (0.66-0.78)	64 (44-104)	386 (304-468)
Period 2							
CH	86.3	5.4	0.602	0.617	0.75 (0.69-0.80)	76 (47-154)	334 (271-397)
FU	8.1	5.2	0.571	0.573	0.69 (0.57-0.80)	10 (7-14)	12 (8-16)
MB	185.2	4.9	0.626	0.618	0.71 (0.59-0.84)	83 (49-193)	995 (677-1313)
ND	4.0	4.5	0.563	0.569	0.76 (0.64-0.88)	16 (9-38)	10 (6-14)
NG	120.1	5.1	0.550	0.572	0.73 (0.67-0.80)	68 (43-130)	549 (439-659)

Population structure and gene flow

During each period, *P. cabanisi* genotypes were assigned to three genetic clusters, corresponding to (i) population CH, (ii) population MB, and (iii) a cluster comprising populations NG, FU and ND (Fig. 2.1, Appendix 2.B). During the second period, the degree of genetic clustering declined (period 1: r = 0.84; 95% CI 0.82-0.86; period 2: r = 0.61; 95% CI 0.57-0.65) in two ways. First, populations NG, CH and MB became more admixed (CH in particular). Second, individuals of populations FU and ND showed a higher likelihood of belonging to the NG cluster, in addition to an increased admixture with MB. Increased genetic admixture over time corresponded with a change in migration-drift equilibrium. While during the first period, 'migration-drift equilibrium' models were 4 times more likely than 'drift only' models ('substantial' evidence for equilibrium), this likelihood increased to 2856 times ('decisive' evidence) during the second period.

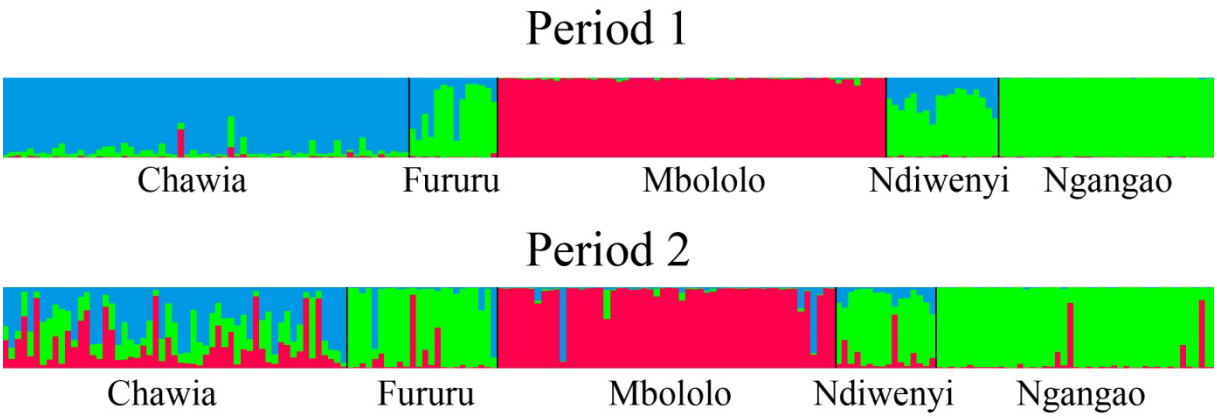

Figure 2.1. Admixture proportions of five *P. cabanisi* populations sampled during two non-overlapping periods (Period 1: 1996-2000; Period 2: 2006-2010) as inferred from Bayesian genetic clustering. Each bar represents an individual sample with color-scales reflecting the likelihood of belonging to one of the inferred genetic clusters.

When pooling all non-focal populations as putative source of migrants, the highest estimated level of gene flow occurred to population FU (m = 0.473) (Table 2.2). Gene flow to each of the large populations was much lower (m = 0.058-0.083) and possibly close to zero in the case of NG (as judged from the 95% CI; Table 2.2). By multiplying estimates of N_{eV} (*open* models) with m, a total of 9 individuals per generation were estimated to migrate to FU (Table 2.2). When treating each of the large populations as separate putative source, an estimated 4.6 immigrants to FU (51%) originated from population NG, as opposed to 2.3 immigrants (26%) from population CH.

Table 2.2. Likelihood estimates of variance effective population size estimated with *open* ($N_{eV\ OPEN}$) and *closed* ($N_{eV\ CLOSED}$) models that assume presence or absence of gene flow (m) among five *P. cabanisi* populations, respectively. Estimates were calculated with MLNE 2.3 under the assumption of a single-generation interval between temporal samples (1996-2000 and 2006-2010). For open models, all populations except the focal one were treated as a single panmictic source of immigrants. N_{eV}*m estimates the number of immigrants per generation. The ratio (N_e/N_c) of the first period is calculated based on the variance effective population size assuming presence of gene flow ($N_{eV\ OPEN}$) – reflecting the effective size of the first period (given that both periods are separated by one generation, see Waples 2005) – and the census population size (N_c) of the first period (see Table 2.1).

Focal population	With gene flow						Without gene flow	
	$N_{eV\ OPEN}$	95% CI	m	95% CI	N_{eV}*m	N_e/N_c	$N_{eV\ CLOSED}$	95% CI
CH	63	(35-277)	0.083	0.018-0.172	5	0.15	132	(51 - >10000)
FU	19	(14-31)	0.473	0.215-0.814	9	1.58	49	(24 - 2266)
MB	64	(34-785)	0.058	0.004-0.13	4	0.08	300	(77 - >10000)
ND	16	(16-41)	0.253	0.079-0.482	4	1.23	68	(20 - >10000)
NG	100	(44-4411)	0.060	<0.001-0.15	6	0.26	122	(53 - >10000)

DISCUSSION

Severe forest fragmentation during the 1960's was earlier shown to have caused strong population fragmentation in a suite of Taita cloud forest bird species, as inferred from spatial patterns of population persistence (Lens *et al.* 2002) and temporal shifts in population connectivity (Callens *et al.* 2011). Building on these results, we here present a detailed demographic and genetic study of five remnant populations of *P. cabanisi* over a 15 year post-fragmentation interval. While individuals were consistently assigned to three genetic clusters, the degree of exchange within and between these clusters increased from a first (1996-2000) to a second (2006-2010) period, as supported by spatially-explicit admixture and migration-drift models (and corresponding F_{ST}/D_{est} values; results not shown). The impact of population NG as main source of immigrants to two small, neighboring populations (FU and ND) also increased with time, while the impact of CH decreased. From dispersal theory, individuals are predicted to show a higher dispersal propensity under habitat saturation than when local vacancies are available (Clobert *et al.* 2001). The respective increase (NG) and decrease (CH) in census population size from the first to the second period thereby likely resulted in territory saturation (NG) versus territory vacancies (CH) in the direction according with the observed dispersal. Earlier, removal experiments by Githiru & Lens (2006) revealed comparable saturation-vacancy dynamics in the sympatric forest-restricted *Pogonocichla stellata*. Alternatively, structural changes in the landscape matrix between NG, FU and ND may have facilitated dispersal during the second period, yet such hypothesis remains speculative in absence of appropriate land-use data. The notion of an overall increase in population connectivity as inferred from genetic data is further supported by the recent colonization of two small forest remnants (MA 2.5 ha; MW 2.3 ha; Fig. 2 General Introduction) in 2005, and subsequent breeding attempts in MA since 2007. Between 1996 and 2004, no *P. cabanisi* had been trapped or observed in either of these fragments, despite equal trapping and search efforts compared to the other fragments.

Apart from the level of genetic admixture, allelic richness and effective population sizes also increased with time. While fluctuations in N_e are not uncommon in natural populations (Miller & Kapuscinski 1997, Heath *et al.* 2002, Waples 2002, Shrimpton & Heath 2003, Fraser *et al.* 2007b, Ruda *et al.* 2010), the fact that estimates remained largely equal or even showed a weak tendency to increase with time in each of the five populations lends support to a temporal trend. Despite N_{eLD} values as low as four (ND) and eight (FU) individuals estimated

from the 1996-2000 samples, i.e. well below the thresholds of 50 and 500 individuals generally assumed to minimize the effects of genetic inbreeding and drift (Franklin 1980, Soulé 1980, Lande 1988, Franklin & Frankham 1998, Lynch & Lande 1998), none of the populations showed evidence for a recent population bottleneck (heterozygote excess). In contrast, two populations (NG and FU) showed lower levels of heterozygosity than expected under an equilibrium SSM model during the second period. While such a heterozygote deficit might result from inadequate sample sizes or the presence of null alleles (Cornuet & Luikart 1996), sample sizes were comparable among populations and there was no indication of null alleles. Rather, the observed deficit is believed to have resulted from an influx of new alleles from genetically distinct populations (in population FU; see similar results in the Vuria population of *Pogonocichla stellata*, Galbusera *et al.* 2004) or from population expansion in absence of strong gene flow (in population NG; see Cornuet & Luikart 1996). The latter scenario was supported by the corresponding increase in N_c in NG from the first to the second period and the strong congruence in N_{eV} estimates obtained from open and closed models across both periods, which is presumed indicative for low gene flow into this fragment (Fraser *et al.* 2007a).

Yet, even when estimates of N_e are based on extensive spatiotemporal genetic data such as in this study, they remain prone to sampling variation, in particular in populations with mixed age structure, overlapping generations, strong gene flow, or when few generations separate temporal samples (Wang & Whitlock 2003, Waples & Yokota 2007, Palstra & Ruzzante 2008, Tallmon *et al.* 2010). In our study, temporal samples (N_{eV}) were only spaced out for one generation, which might have caused N_e estimates to vary in diverse and complex ways (i.e. depending on patterns of population-specific survivorship and age classes sampled; Luikart *et al.* 2010). Still, adult survival rates did not differ between populations nor periods, and equal random mixtures of all age classes were sampled during both time periods. For single sample models, N_{eLD} estimates are believed to be relatively accurate provided that random, mixed-aged samples include a number of consecutive age classes, approximately equal to a generation length, and populations are not very large (Jorde & Ryman 2007, Waples & Do 2010). The fact that estimates of N_{eLD} were comparable among the three large populations and yielded slightly smaller estimates than temporal-based estimators in both small populations, suggest that potential biases associated with small sample sizes were probably small too.

Ratios of effective to census population sizes (N_e/N_c) in the large populations (0.08-0.26) corresponded well with common estimates in natural bird populations (i.e. 0.1-0.5; Nunney 1993, Frankham 1995, Brown *et al.* 2007, Palstra & Ruzzante 2008). In contrary, rates calculated in the smallest populations FU and ND were highly inflated (both > 1; see Table 2.2). The high N_e/N_c ratios in these small populations most probably resulted from an increase in gene flow (mainly from NG and MB) while their census population sizes remained largely stable throughout the study. Alternatively, inflated N_e/N_c values might have resulted from stochastic biases in N_c estimation, as has been shown in small butterfly populations from stochastic environments (Saarinen *et al.* 2009). However, this scenario is deemed unlikely given the longevity of *P. cabanisi* (oldest Taita individual known to be alive for at least 13 years), and the multiple-year mark-recapture program on which N_c estimates were based. Another option is that the high N_e/N_c ratios in these small fragments resulted from a decrease in the standardized variance in family size as N_c decreases (Hedrick 2005). As such, highly reduced adult population sizes may result in more offspring from more reproductive pairings surviving to maturity. In salmonid fishes, such 'genetic compensation' mechanism has been associated with particular breeding properties that increase fertilization success at low breeding densities (Ardren & Kapuscinski 2003, Araki *et al*. 2007). Whether, and to what extent, such mechanism may also operate in fragmented populations of a group-living species such as *P. cabanisi*, for instance through reduced numbers of non-breeding helpers, remains a topic of further research.

In conclusion, spatiotemporal analysis of genetic and demographic data over a recent fifteen year time span did not provide evidence for increased genetic erosion and isolation in fragmented populations of *P. cabanisi*, but rather showed increased levels of genetic variation, effective population sizes, and genetic admixture. It is currently unclear which ecological or evolutionary factors may have triggered such population responses. As part of a series of conservation initiatives, over 150000 indigenous tree seedlings have been raised in community-owned tree nurseries and planted within indigenous forest remnants and the Taita landscape matrix since 2006 (Githiru *et al.* 2011). While it is tempting to believe that these actions underlie the observed changes in genetic population structure in *P. cabanisi*, such a conclusion remains uncertain given the short timeframe involved. Also, given the diminutive census population sizes in the small forest remnants, this species and ecologically-equivalent ones likely remain prone to demographic and environmental stochasticity. However, from a conservation perspective, findings from this study are promising in the fact that increased

connectivity at metapopulation level seem to dampen demographic and genetic effects of forest loss and degradation at the local population level.

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CONTRIBUTIONS

While historical samples (blood and feathers) were obtained from previous research activities (Taita Hills Biodiversity Project), all field data for the contemporary time period were collected by T. Callens, thereby assisted by a ringing team of two field assistants: L. Wagura (qualified ringer from the National Museums of Kenya) and L. Chovu (local field worker). DNA-extraction and genotyping were executed by V. Vandomme. T. Callens analyzed all genetic data, with assistance of E.Y. Durand for the clustering analyses in TESS. C. Hallmann performed the mark-recapture analyses in MARK and C. Vangestel provided statistical advice. T. Callens wrote the chapter, assisted by L. Lens, M. Githiru and E. Matthysen during the writing phase.

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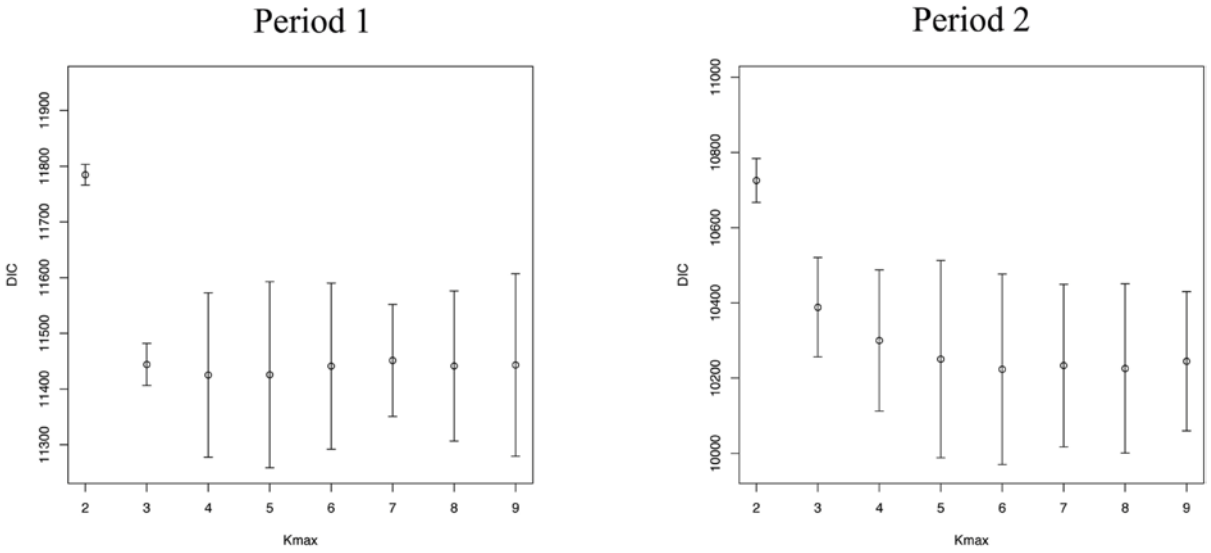
APPENDICES

Appendix 2.A. Multiplex sets, origin, DNA specifications and primermix concentrations of ten microsatellite DNA markers for *P. cabanisi*.

Multiplex	Locus	Species[1]	Reference	Product size (bp)	Primermix conc (µM)
1	Ls1	*Lanius ludovicianus*	Mundy & Woodruff 1996	150-230	0.2
1	Mcyµ4	*Malurus cyaneus*	Double *et al.* 1997	127-162	0.2
1	Pca3	*Parus caeruleus*	Dawson *et al.* 2000	150-179	0.2
1	Pca4	*Parus caeruleus*	Dawson *et al.* 2000	125-180	0.4
2	Indigo41	*Vidua chalybeata*	Sefc *et al.* 2001	260-315	0.1
2	Ase18	*Acrocephalus sechellensis*	Richardson *et al.* 2000	221-360	0.14
2	WBSW2	*Plocepasser mahali*	McRae & Amos 1999	207-241	0.3
3	Ls2	*Lanius ludovicianus*	Mundy & Woodruff 1996	188-203	0.2
3	Pfi04	*Phyllastrephus cabanisi*	R.C.K. Bowie, unpublished data	134-207	0.1
3	Pfl54	*Phyllastrephus cabanisi*	R.C.K. Bowie, unpublished data	220-276	0.4

[1]species for which the primer was originally developed

Appendix 2.B. Bayesian admixture model selection in TESS 2.3 for two non-overlapping time periods (Period 1: 1996-2000; Period 2: 2006-2010). DIC values were plotted against K-values (maximal number of clusters) and K-values corresponding to DIC values that first levelled off, have been selected.



Cabanis's greenbul (*Phyllastrephus cabanisi*) breeding in the Taita Hills. © Alexander Callens

Avian nest predation strongly varies with timing of nest initiation, concealment and distance to edge in a fragmented Afrotropical forest

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ABSTRACT

High levels of nest predation shape the population dynamics of many tropical birds, especially when deforestation alters nest predator communities. We studied predation on natural nests of the cooperatively breeding cabanis's greenbul (*Phyllastrephus cabanisi*) during three consecutive breeding seasons in a highly fragmented cloud forest in SE Kenya. Overall predation rates derived from 223 nests averaged 70%, matching the typical high predation level on tropical species. However, predation rates strongly varied in space and time, and a model that combined habitat-, edge- and timing effects was best supported by our data. Nest predation rates consistently increased from forest edge to interior, opposing the classic edge effect on nest predation. Nest concealment and vegetation structure also affected predation rates, but the strength and direction of the relationships varied across years and fragments. Apart from spatial variation, predation rates also declined during the breeding season. Complex and variable relationships with nest predation, such as demonstrated here, suggest that several underlying mechanisms interact and imply that fixed nesting strategies may have variable - even opposing - fitness effects between years and habitats.

INTRODUCTION

Predation on eggs and nestlings constitutes a major cause of reproductive failure in birds (Wilcove 1985, Martin and Roper 1988, Sieving 1992), and high predation rates have been shown to trigger avian population decline (Martin and Clobert 1996, Stratford and Robinson 2005). Understanding spatial and temporal variation in nest predation is therefore a prerequisite to predict avian population dynamics and viability under landscape or climate change (Robinson *et al.* 1995, Chalfoun *et al.* 2002, Grant *et al.* 2005). This particularly applies to species from tropical rainforests that currently suffer from severe anthropogenic effects such as habitat loss and biological invasions, without similar episodes in the recent past. The ensuing absence of past extinction filters (Hannah *et al.* 1995, Balmford 1996), in combination with high levels of ecological specialization (Henle *et al.* 2004, Brook *et al.* 2008), renders tropical species highly vulnerable to contemporary selective pressures. Yet, data on tropical nest predation remain scarce and are strongly biased towards Neotropical forests (e.g. Martin *et al.* 2000, Robinson *et al.* 2000, Kvarnbäck *et al.* 2008, Ryder *et al.* 2008, Young *et al.* 2008, Brawn *et al.* 2011; but see Thompson 2004, Spanhove *et al.* 2009a, Newmark and Stanley 2011 for examples on Afrotropical forest birds).

Reproductive failure due to nest predation is generally high in small, isolated and degraded forest fragments (Small and Hunter 1988, Tewksbury *et al.* 2006), in particular near forest-landscape ecotones (edge effect on nest predation *sensu* Gates and Gysel 1978, Andrén and Angelstam 1988, Lahti 2001, Batáry and Báldi 2004). Ecological edge effects have earlier been linked to at least three complementary processes. First, the high structural diversity at habitat ecotones may provide foraging and nesting opportunities for a wide range of bird species. This can result in higher nest densities and attraction of more (diverse) nest predators (Gates and Gysel 1978, Weldon and Haddad 2005). Second, generalistic predators from the non-forest landscape matrix may invade the forest edges (Andrén and Angelstam 1988). Third, barriers between forests and open areas may act as ‘travel lanes’, also resulting in increased predator activity (Bider 1968, Ferguson 2000), although the empirical evidence for this hypothesis remains poor (Larivière 2003). Apart from these edge-related effects, nest predation rates have also been shown to vary with nest concealment, nest height and canopy structure (Martin 1992, 1993, Lambert and Kleindorfer 2006, Colombelli-Négrel and Kleindorfer 2009), but the impact of these characteristics strongly differs across study sites.

In addition to spatial variation, nest predation rates may also vary with timing of breeding (Mezquida and Marone 2001, Peak 2007) and among successive stages of the breeding cycle, i.e. egg laying, incubation, and nestling feeding (Peak *et al.* 2004, Grant *et al.* 2005, Brawn *et al.* 2011). While some studies revealed higher predation rates during egg laying (Mezquida and Marone 2001), others held increased parental and nestling activity responsible for higher rates recorded during the nestling stage (Redondo and Castro 1992, Haff and Magrath 2011). Some studies found more complex relationships, such as decreased predation during egg-laying, increased predation during incubation, and decreased predation after hatching (e.g. cubic or saw-tooth relationships; Grant *et al.* 2005, Low and Pärt 2009). Such variable patterns likely reflect underlying variation in the abundance, activity patterns, prey selection and/or functional responses of predators - factors that are generally considered more important than nest-related factors in explaining variation in predation rates (Thompson and Nolan 1973, Peak 2003, Kvarnbäck *et al.* 2008, Sperry *et al.* 2008). Because nest survival models that assume constant predation risk throughout the nesting cycle may hence produce biased survival estimates, models have been developed that can accommodate temporal variation in predation risk (e.g. Stanley 2000, Dinsmore *et al.* 2002, Shaffer 2004).

Using such an approach, we here test if, and to what extent, predation rates on natural nests of a cooperatively breeding forest bird vary with distance to the forest edge, vegetation structure, timing of breeding and breeding stage, during three consecutive years in two isolated cloud forest fragments in south-east Kenya. Like most other tropical species, cabanis's greenbuls (*Phyllastrephus cabanisi*) tend to lay smaller clutches, incubate their eggs longer, provide longer post-fledgling care, and show higher adult survival rates compared to temperate forest birds of comparable size and ecology (Martin 1996, Stutchbury and Morton 2001, information for *P. cabanisi* from Keith *et al.* 1992 and T. Callens *unpubl. data*). Because tropical nest predator guilds are highly diverse (Skutch 1985, Roper and Goldstein 1997, Robinson and Robinson 2001) and small forest fragments typically suffer from elevated predator densities, we expected very high nest predation rates in disturbed tropical forests. On the other hand, the anti-predator behavior of this social species may relax predation compared to sympatric species. Given the strong heterogeneity in strength and direction of relationships with nest predation emerging from the literature, we here apply an information-theoretic approach to select the most parsimonious model(s) from a set of fifteen logistic-exposure models (*sensu* Shaffer 2004), without making *a priori* predictions on single factor effects.

MATERIAL AND METHODS

Study area

The Taita Hills (maximum altitude 2220 m) are located in south-east Kenya (03°24'S, 38°21'E), on the dry Serengeti plains that isolate these hills from other highland blocks by over 80 km in either direction (Lovett 1985, Pellikka *et al.* 2009). While long (Mar-May) and short (Nov-Dec) rainy seasons alternate within the Intertropical Convergence Zone, mist and cloud precipitation is a year-round phenomenon in the Taita forests. The verdant Taita Hills constitute the northernmost extension of the Eastern Arc Mountains, and with over 50% indigenous forest loss since 1955 (Pellikka *et al.* 2009), this archipelago is ranked among the most threatened sites in this globally important biodiversity hotspot (Lovett and Wasser 1993, Myers *et al.* 2000). At present, the Taita forest archipelago covers 430 ha of indigenous forest, fragmented into three larger forest patches (86-185 ha) and eight tiny remnants (2-8 ha) (Pellikka *et al.* 2009), embedded in a densely populated mosaic of small-scale subsistence agriculture, bushes and exotic plantations. The remaining forest patches are mostly of a degraded Afromontane type, characterized by *Albizia gummifera*, *Macaranga conglomerate, Newtonia buchananii, Phoenix reclinata, Strombosia scheffleri, Tabernaemontana stapfiana,* and *Xymalos monospora* (Chege and Bytebier 2005, Aerts *et al.* 2011).

Nest data for this study were collected in two of the larger forest remnants, i.e. Chawia forest (CH, 86 ha) and Ngangao forest (NG, 120 ha). CH is located at the top of a gently sloping cliff (1470-1600 m), while NG is located ca. 11 km north of CH, on an eastern slope (1700-1952 m) of a north-south oriented mountain ridge (Fig. 2 General Introduction). CH is considered to be much more intensively disturbed compared to NG, due to its long history of forest clearing, establishment of exotic stands and strong human-induced disturbance (Beentje 1987, Wilder *et al.* 1998, Chege and Bytebier 2005, Pellikka *et al.* 2009). During 956 hours (CH) and 1650 hours (NG) of monitoring of trespassing for firewood collection, pole cutting and cattle grazing (breeding seasons of 2008-09 and 2009-10), a total of 144 (CH) and 26 (NG) encounters with humans were recorded, reflecting a tenfold higher incidence per time unit in CH. Based on visual observations and video-recordings (2007-2010) at *P. cabanisi* nests, the following nest predators were encountered: *Accipiter tachiro* (African goshawk), *A. melanoleucus* (Black goshawk), *Cercopithecus mitis albogularis* (Sykes's Monkey, Photo 3.1A*)* and *Papio cyanocephalus* (Yellow baboon). We refer to Spanhove *et al.* (2009a) for a

more comprehensive list of putative nest predators in the Taita Hills, comprising mammals [rodents (e.g. Photo 3.2B), shrews, civets & mongooses], reptiles (snakes) and birds.

Photo 3.1. Pictures of confirmed predators of *P. cabanisi* nests in the indigenous forests of the Taita Hills: (A) Sykes's monkey (*Cercopithecus mitis albogularis*) and (B) Red-legged sun squirrel (*Heliosciurus rufobrachium*). © Alexander Callens

Study species

P. cabanisi is a forest specialist of central- to east-African (sub)tropical lowland to montane moist forest (Keith *et al.* 1992, Bennun *et al.* 1996). Globally, the IUCN conservation status of the species is currently evaluated as 'Least Concern' (Birdlife International 2012).Within the Taita forest archipelago, the species has been recorded in eleven indigenous forest fragments, where noisy flocks forage in the understory and near *Dorylus* ant swarms (Keith *et al.* 1992). In case of disturbance by humans or predators, parents and co-breeders perform distraction moves such as flicking tails and wings, producing alarm calls and rattling away from the nest, and even attacking potential predators (Keith *et al.* 1992, T. Callens *pers. obs.*). The species builds cup-shaped nests at an average height of 1.3 m in shrub or small tree species such as *Chassalia discolor, Culcasia scandens, Dracaena steudneri* and *Uvaria sp.* The onset of breeding coincides with the start of the late rain season and the breeding season generally runs from mid October till the end of March (Keith *et al.* 1992). Most clutches contain 2 eggs (15 x 22 mm), which are incubated during 17 days and most nestlings fledge after 11-13 days. Adult mates remain paired during multiple seasons and video-recordings showed that helpers assist adult territory owners in raising their offspring (see Chapter 4). Based on long-term mark-recapture data, *P. cabanisi* shows high site fidelity and a long lifespan (oldest individual known to be alive for at least 13 years). Compared to six sympatric forest bird species, *P. cabanisi* appears moderately sensitive to indigenous forest disturbance (Lens *et al.* 1999), while a comparison of historic population differentiation (estimated from

microsatellite genotypes) with contemporary dispersal rates (estimated from capture-recapture data) indicated moderate loss of mobility over time (Callens *et al.* 2011).

Nest monitoring

During three consecutive breeding seasons (2007-2008, 2008-2009, 2009-2010), six experienced observers searched all suitable habitat and used behavioral clues to locate as many *P. cabanisi* nests as possible in both forest fragments. Once detected, nest fate was recorded every third day on average, allowing analysis based on daily predation rates *sensu* Mayfield (1961). Nests observed during the building phase only, or initially detected with fully-grown fledglings expected to fledge the same day, or with causes of failure other than predation, were omitted from further analysis.

All detected nests were located within the indigenous forest boundaries, so for each nest, the distance to the nearest indigenous forest boundary was calculated in Arcmap 9.2 (Environmental Systems Research Institute, 1999-2006) using a combination of GPS coordinates (Garmin GPSMAP60CSx, root mean square error of 6m under canopy) and a forest boundary vector layer based on recent aerial photographs (25.01.2004, Pellikka *et al.* 2009) and land cover data derived from a SPOT 4 image (15.10.2003, Clark and Pellikka 2009). Exotic plantations and large open areas within the forest were not considered as indigenous forest. The following vegetation-related variables were recorded: nest concealment (i.e. percentage covered by foliage at a distance of 1 m) was estimated from six directions (above, below and the four cardinal directions, see Remeš 2005, Lambert and Kleindorfer 2006), and the percentage of canopy cover was derived from standardized canopy pictures taken at each nest (NIKON D100 digital camera with a wide angle lens; pictures taken at an average height of 1.5m, Photos 3.3A&D). For the latter, an “Isodata” classification was performed in ImageJ ver.1.41o, using the blue band (Photos 3.3B&E) to maximize the contrast between foliage and sky (Pellikka 2001, Photos 3.3C&F). Because these vegetation variables were not independent, a principal component analysis was performed to avoid collinearity (Proc Princomp in SAS 9.2, SAS Institute Inc. 2008). The first component (PC1) explained 27% of the variance (see Appendices 3.A&B for details on the PCA-results) and corresponded with a strong gradient from poorly concealed nests under closed canopy forest to highly concealed nests under a more open canopy forest. This principal component was used as vegetation parameter in further analyses. To study temporal variation in nest predation, we inferred the onset of egg laying (clutch initiation date) either from the last

observation of a nest under construction, from the first and/or last observation date with intact eggs, or from the age of the nestlings (see also Spanhove *et al.* 2009a). Clutch initiation date was subsequently used to estimate the nest age and stage, and was used to assess relationships between the timing of breeding and nest predation.

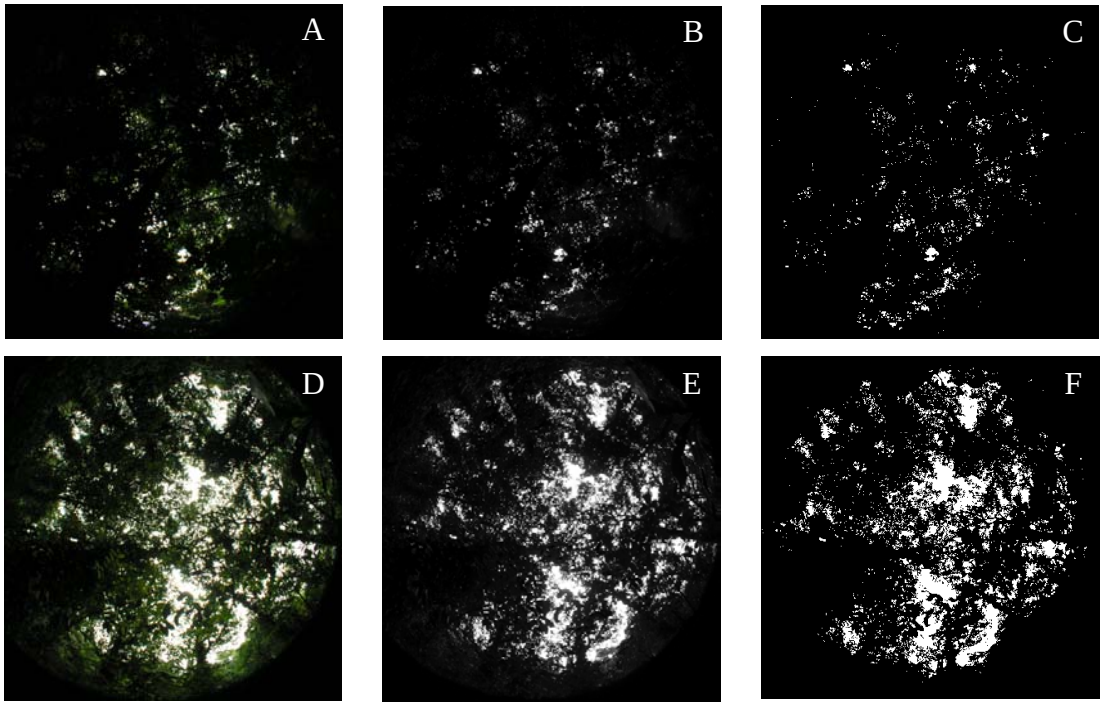

Photo 3.3. Processing of canopy pictures to calculate the percentage of canopy cover showing the original pictures (A&D), the blue band selection (B&E) and the black-and-white contrast result (C&F) for closed (upper row) and more open (lower row) canopy cover.

Statistical analysis

Nest predation rates were analyzed with logistic-exposure models, which are generalized linear models with binomial distribution and a link function designed for flexible nest predation analysis based on daily predation rates. More specifically, these models account for the fact that survival probability depends on the interval length by defining the logistic-exposure link function as $\log_e[\theta^{1/t} / (1-\theta^{1/t})]$, with θ the daily survival rate and t the interval length (in days) between two nest controls (Shaffer 2004). We *a priori* defined fifteen potential models in which the explanatory variables were grouped according to putative spatial and temporal sources of variation (Table 3.3). In none of these models, overdispersion was observed. All models were ranked and weighted based on a second order Information Criterion (AICc; Burnham and Anderson 2002). As the most parsimonious model was clearly superior to all alternative ones (see Table 3.3 in results), inference was made from this model

only, rather than from an averaged "unconditional" model. Analyses were performed using Proc Genmod in SAS 9.2 (SAS Institute Inc. 2008).

RESULTS

A total of 223 active nests were monitored (54 nests in 2007-2008, 84 nests in 2008-2009, 85 nests in 2009-2010, see Figures 3.1&3.2, Tables 3.1&3.2), of which the majority was discovered at an early stage of nest development (90 in the building stage, 109 in the egg stage and 24 in the nestling stage). Overall, 124 of these nests were predated, corresponding to an apparent predation rate of 56%. Frequent nest controls resulted in 1126 time intervals checks in which the nest fate was recorded. From these data, the logistic-exposure model estimated an average daily predation rate of 0.038, corresponding with an overall predation rate of 70% (±SE: 67-73%) after 30 days.

Table 3.1. Number of nests found in different stages, seasons and fragments.

	Chawia			Ngangao		
	2007-2008	2008-2009	2009-2010	2007-2008	2008-2009	2009-2010
building stage	13	10	13	12	14	28
egg stage	10	20	13	12	30	24
nestling stage	3	1	2	4	9	5
Total	26	31	28	28	53	57

Table 3.2. Number of nests predated (total observation days in between brackets) in different stages, seasons and fragments.

	Chawia			Ngangao		
	2007-2008	2008-2009	2009-2010	2007-2008	2008-2009	2009-2010
egg stage	3 (175 d)	7 (217 d)	7 (260 d)	7 (180 d)	14 (284 d)	23 (436 d)
nestling stage	6 (320 d)	8 (312 d)	5 (250 d)	14 (230 d)	20 (397 d)	10 (378 d)
Total	9 (395 d)	15 (529 d)	12 (510 d)	21 (410 d)	34 (681 d)	33 (814)

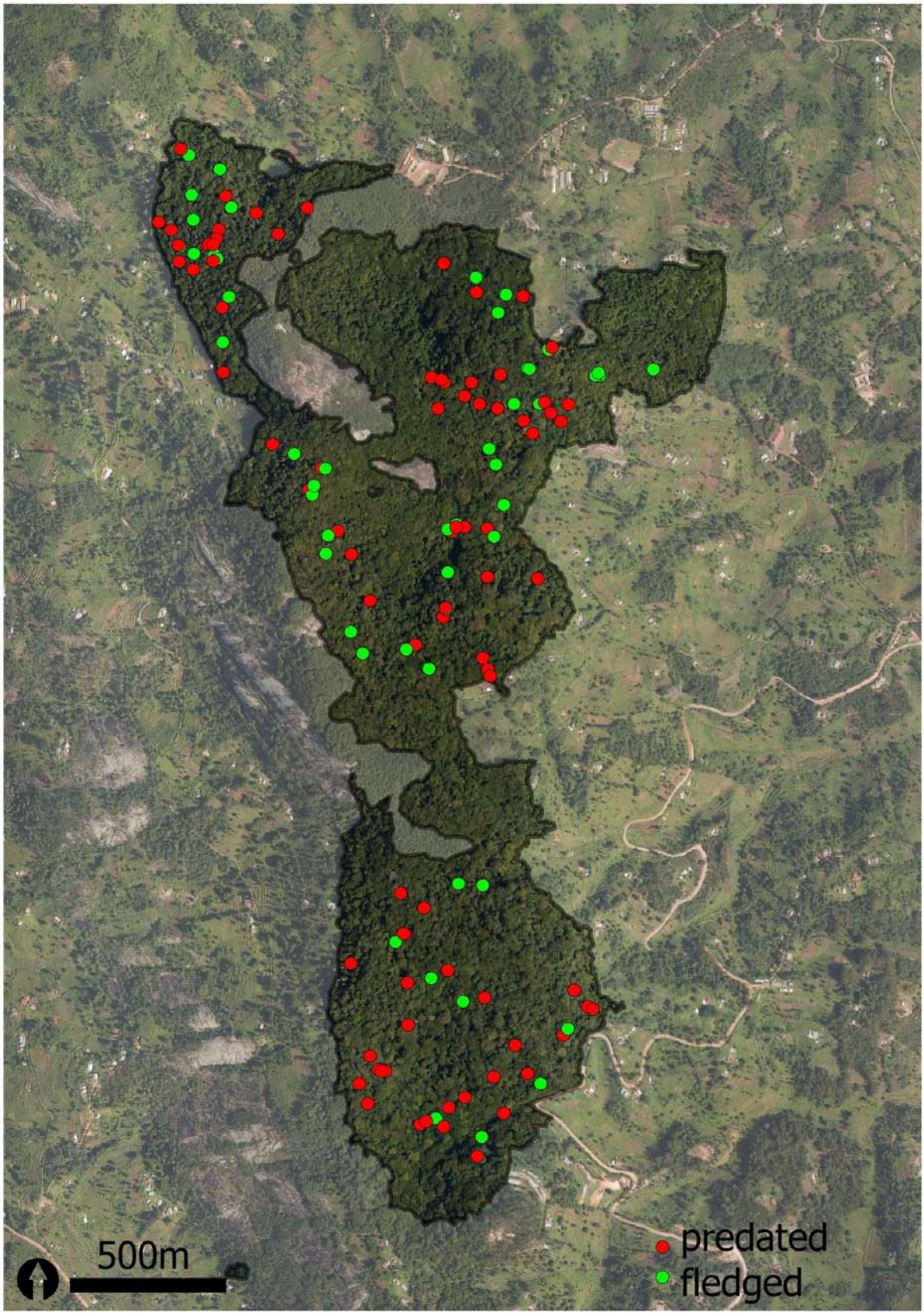

Figure 3.1. Detailed aerial photograph of Ngangao indicating the indigenous forest area and locations of all failed (predated) and successful (fledged) nests.

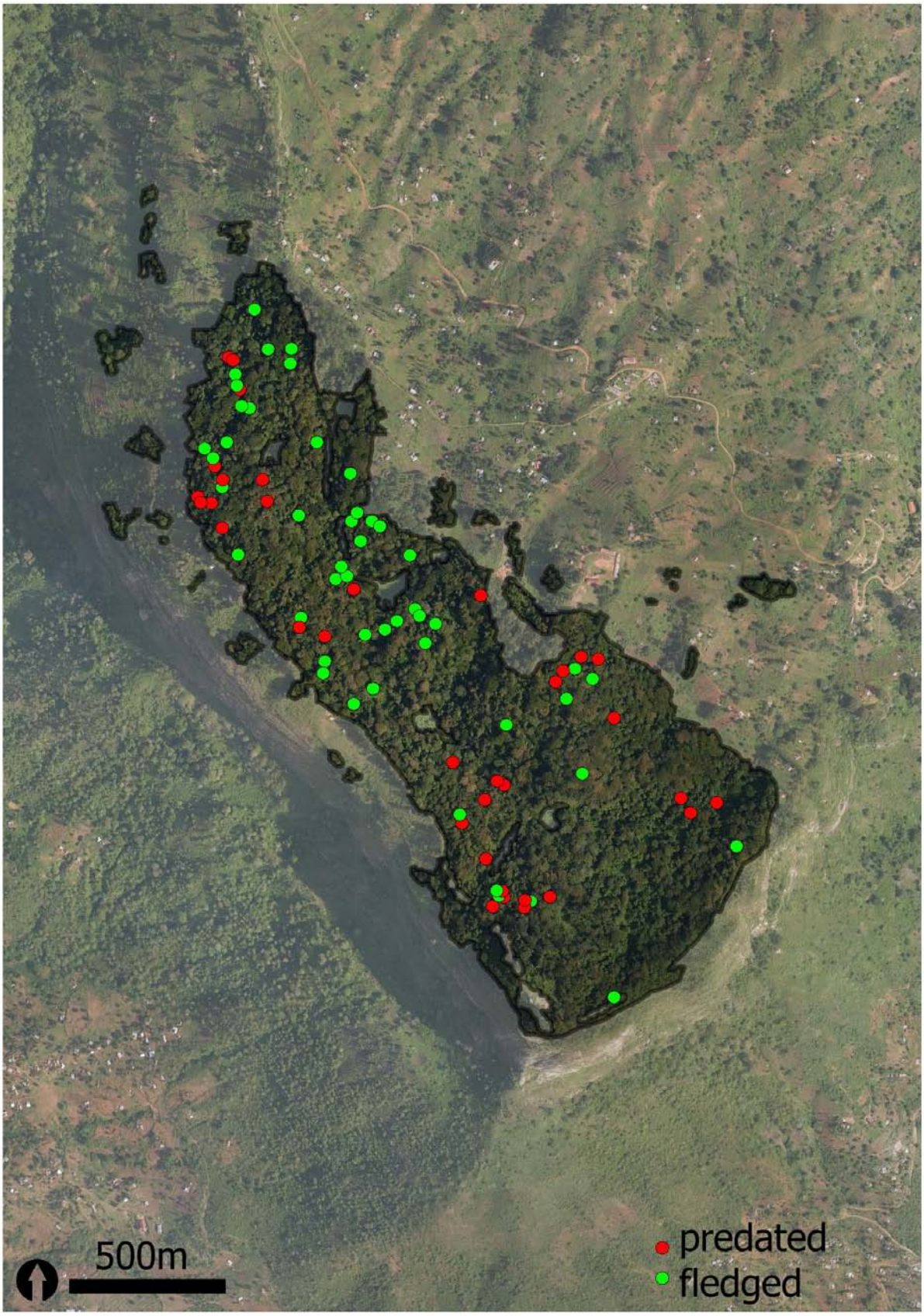

Figure 3.2. Detailed aerial photograph of Chawia indicating the indigenous forest area and locations of all failed (predated) and successful (fledged) nests.

Predation rates strongly varied among nests. The two most parsimonious models comprised both temporal and spatial effects (Table 3.3; see Appendix 3.C for parameter estimates), indicating that predation on *P. cabanisi* nests differed between the two fragments and depended on the combined effect of clutch initiation date and distance to the edge. At a finer spatial scale, vegetation structure and canopy cover also affected nest predation rates, however, only in models that accounted for variation at larger scales. Models with fewer parameters were less strongly supported by our data, suggesting that nest predation cannot be explained by a few key characteristics. Rather, a suite of variables seems to predict spatial and temporal variation in predation rates on natural nests of *P. cabanisi*. A difference in daily predation rate on nests with eggs or nestlings was not supported by our data (Table 3.3).

Table 3.3. Fit statistics of 15 *a priori* defined statistical models that explain spatial and temporal variation in daily predation rates on *P. cabanisi* nests in two cloud forest fragments. Models are compared based on the number of parameters (k), Akaike's Information Criterion corrected for sample size (AICc), difference in AICc from the best-fitting model (ΔAICc), and model weight (W_j) indicating the relative support for each model.

Model	k	AICc	ΔAICc	W_j
LANDSCAPE [a] + TIMING [b] + VEGETATION [c]	**11**	**727,3**	**0,0**	**0,80**
LANDSCAPE [a] + TIMING [b]	9	730,9	3,6	0,13
LANDSCAPE [a] + VEGETATION [c]	6	734,1	6,8	0,03
LANDSCAPE [a]	4	735,3	7,9	0,02
LANDSCAPE [a] + STAGE [d] + VEGETATION [c]	9	736,0	8,7	0,01
FRAGMENT	2	736,1	8,8	0,01
LANDSCAPE [a] + STAGE [d]	7	737,3	9,9	6,E-03
DISTANCE TO FOREST EDGE	2	742,1	14,8	5,E-04
NULL MODEL	1	748,6	21,3	2,E-05
STAGE [d]	4	750,1	22,7	9,E-06
VEGETATION	2	750,6	23,2	7,E-06
SEASON	3	751,6	24,2	4,E-06
STAGE [d] + VEGETATION	5	752,1	24,8	3,E-06
TIMING [b]	6	754,5	27,2	1,E-06
TIMING [b] + VEGETATION	7	756,4	29,0	4,E-07

[a] comprises FRAGMENT, DISTANCE TO FOREST EDGE, and the two factor interaction
[b] comprises CLUTCH INITIATION DATE, SEASON and the two factor interaction
[c] models with LANDSCAPE and VEGETATION effects include the FRAGMENT*VEGETATION interaction
[d] comprises NEST AGE, NEST STAGE and the two factor interaction

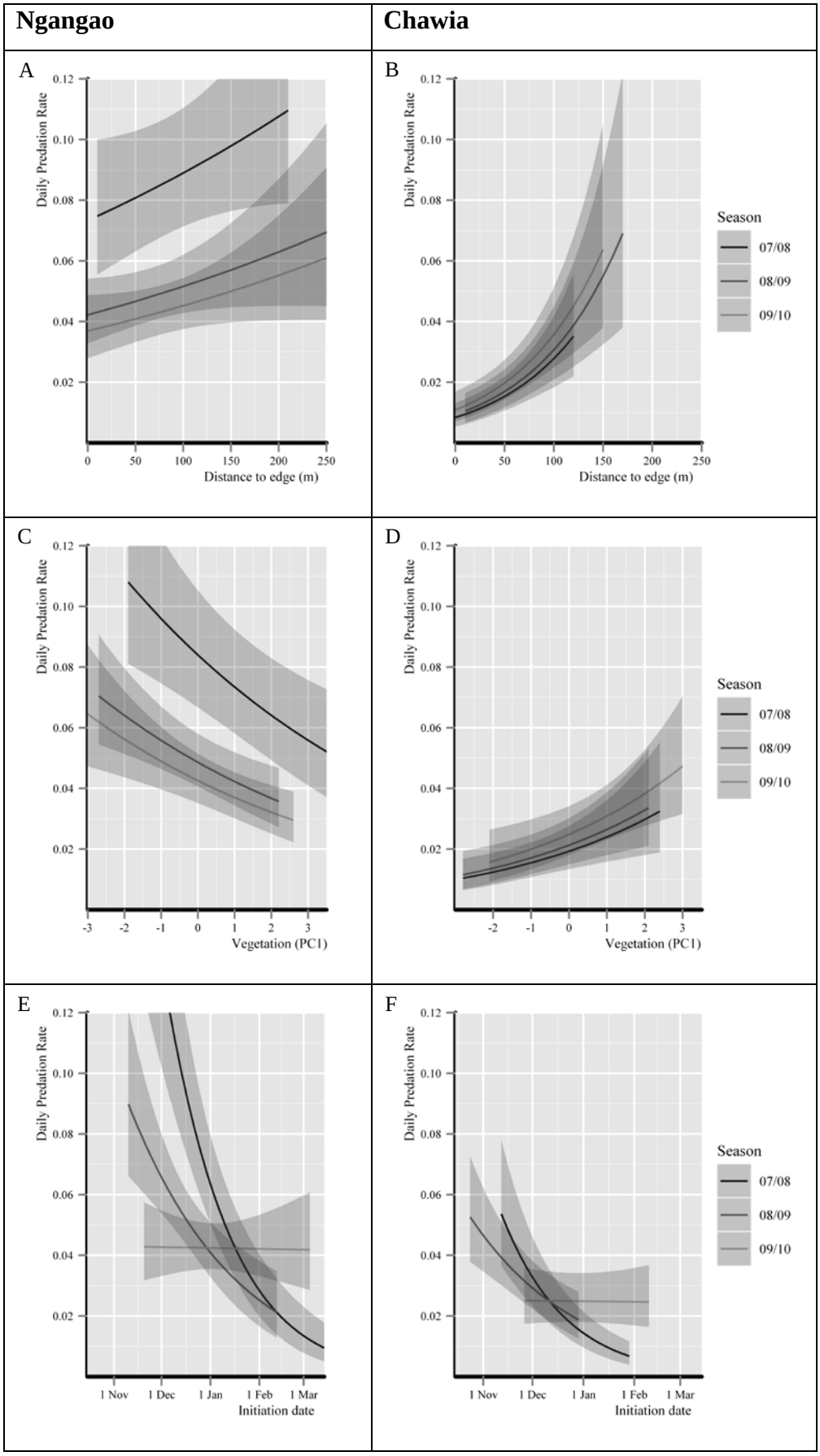

Figure 3.3. Daily predation rates on *P. cabanisi* nests in relation to (i) distance to forest edge in fragment NG (A) and CH (B); (ii) vegetation characteristics (PC1 of nest concealment and canopy cover, corresponding with a gradient of poorly concealed nests under closed canopy forest to highly concealed nests under more open canopy forest) in fragment NG (C) and CH (D); and (iii) clutch initiation date in fragment NG (E) and CH (F). Estimates (± SE) are based on the most parsimonious model and restricted to the observed range of the explanatory variable.

The most parsimonious models supported an increase in nest predation towards the interior of both forest fragments. In fragment NG, daily predation rates nearly doubled from the edge towards the interior (Fig. 3.3A), while a six-fold increase was detected in fragment CH (Fig. 3.3B). Vegetation structure also affected nest predation rates, but with different effects in each of the fragments. In NG, nest predation decreased with increasing PC1, indicating that predation levels were highest for poorly concealed nests under closed canopy (Fig. 3.3C), while in CH, the opposite pattern was found (Fig. 3.3D). In NG, nest predation rates tended to be more strongly affected by vegetation structure than by edge effects (a three- versus twofold change, respectively). In CH, predation rates increased only 1.5 times from less concealed nests under a closed canopy to well concealed nests under more open canopy, compared to six times due to inverse edge effects. In both fragments, timing of breeding had the strongest effect on nest predation: nests initiated early in the breeding season had a higher probability to become predated compared to later ones (Figures 3.3E&F). However, this pattern strongly varied between years as shown by a tenfold decrease in the first year compared to no trend in the last one.

DISCUSSION

Results from this study show that on average 70% of all initiated clutches of *P. cabanisi* were predated at the egg or nestling stages. This rate corresponds with the average predation rate on tropical birds (Robinson *et al.* 2000, Stutchbury and Morton 2001), despite the anti-predator behavior expected to decrease nest predation. Predation rates in the Taita Hills were, however, much lower than those recorded on a suite of forest dependent species in a nearby area of the Eastern Arc Mountains with comparable landscape structure and forest ecology (East Usambara Mountains, Tanzania). For *P. cabanisi*, daily predation rates in our study area were nearly two times lower than those recorded in continuous Usambara forest, and nearly six times lower than in Usambara's forest fragments (Newmark and Stanley 2011). Within the Taita Hills, nest predation was highly variable in space and time. Rates consistently increased from the forest edge toward the interior, a finding that strongly opposes the predictions from 'classic' edge effects on nest predation (*sensu* Gates and Gysel 1978, Lahti 2001). Higher predation in the forest interior rather supports the notion of an 'inverse' edge effect, as earlier observed on nests of *Pogonocichla stellata* in the Taita Hills (Spanhove *et al.* 2009a), and of a small number of other species elsewhere (Lahti 2001, Newmark and Stanley 2011). Inverse edge effects on nest predation can be triggered by at least two different mechanisms. First, relaxed predation rates near forest edges may reflect low local abundances of native (ground-dwelling) nest predators, possibly due to competition from, or predation by, domestic or feral predators (Maina and Jackson 2003). At the same time, meso-predator release (*sensu* Crooks and Soulé 1999) due to the loss of primary predators from small or degraded forest fragments may cause high predation rates in the forest interior, the combined effect of which may result in inward gradients in predation risk. While domestic dogs were abundant near forest edges in our study area, their impact on predator populations of *P. cabanisi* (mainly arboreal) remains unknown. Alternatively, inverse edge effects may result from a preference for forests interior habitat by nest predators, either because of higher habitat quality, lower human impact, or both (Carlson and Hartman 2001, Spanhove *et al.* 2009b). We assume that for the shrub-nesting *P. cabanisi*, such mechanism is more likely to explain the observed pattern in nest predation as the only predators confirmed from visual observations and videotaping (two raptors, *Accipiter tachiro* and *A. Melanoleucus* and two monkeys, *Cercopithecus mitis* and *Papio cyanocephalus)* are mostly recorded in the interior of indigenous forest fragments, and monkeys are chased by farmers surrounding the fragments. Formal discrimination between these alternative mechanisms, however, requires targeted population studies on a wider suite

of nest predators. Irrespective of these (or other) underlying mechanisms, strongly reduced levels of nest predation in more degraded forest patches or closer to forest edges may ultimately result in increased breeding success of small forest passerines in severely fragmented landscapes (Spanhove *et al.* 2009a).

Apart from edge-related variation, predation rates in fragment NG were also lower in more concealed nest, most likely because visual, chemical or auditory cues that attract nest predators were more strongly masked (Martin 1993, Caro 2005, Colombelli-Négrel and Kleindorfer 2009). Surprisingly, an opposite trend was observed in fragment CH, with more concealed nests suffering from higher predation levels. Such unexpected effect may result from the fact that high levels of nest concealment are often indicative of dense understory habitat, where nest predators such as rodents and other small mammals can thrive. Abundances of these species have been shown to be higher in CH compared to NG (Odhiambo 2000, Oguge *et al.* 2004), although evidence for nest predation by either of them is currently lacking, nor do we have accurate density estimates to directly test this hypothesis.

Besides spatial variation, predation rates on *P. cabanisi* nests also showed strong temporal variation, both within and between breeding seasons. The most striking finding was a steep decrease in predation rates during the course of the first two breeding seasons. Such pattern opposes the more widely supported pattern of increased predation rates with time resulting from shifts in predator abundance, activity patterns or search efficiency (Mezquida and Marone 2001, Grant *et al.* 2005, Peak 2007), although decreasing trends have also been observed for some temperate bird species (Peak 2003, Shustack and Rodewald 2011). Likewise, predation rates were not higher during the nestling stage compared to egg stage, contrary to many other studies (e.g. Martin 1992, Peak *et al.* 2004, Grant *et al.* 2005, Brawn *et al.* 2011), despite the fact that *P. cabanisi* nests were more frequently visited by parents and helpers after the eggs hatched (T. Callens *unpubl. data*). However, our nest observations also showed that *P. cabanisi* nestlings do not show begging behavior, thereby reducing the relative difference in predator attraction between nests with eggs or nestlings.

Spatial and temporal variation in the strength or direction of relationships with nest predation suggests that predator communities, predator densities, or predator strategies may vary in space and time. As an example, for generalist (omnivorous) predators that also feed on fruits and seeds, phenological differences in fruit setting between fragments and years, a well-

known phenomena in tropical fragmented landscapes (see Lehouck *et al.* 2009 for a study in the Taita archipelago), may affect their dependency on eggs or nestlings. Complex and variable relationships with nest predation, such as demonstrated here, suggest that several underlying mechanisms interact and imply that fixed nesting strategies may have variable (and even opposing) fitness effects between years and habitats.

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CONTRIBUTIONS

Intensive nest searching was executed by six experienced local field workers (M. Chovu, L. Chovu, A. Mwakulomba, P. Kafusi, I. Mwashighadi and N. Mkombola). T. Callens supervised these search activities in the field, personally executed all nest checks after initial detection (until predation/fledging event) and ringed/measured all nestlings. Concerning these activities, T. Callens was temporarily assisted by master students (C. Hallmann, S. Piirainen), a local field assistant (M. Chovu) and a volunteer (A. Callens). T. Callens and T. Spanhove analyzed the data and wrote the article in concert. L. Lens, C. Hallmann and P. Pellikka assisted throughout the writing phase.

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APPENDICES

Appendix 3.A. Results of the Principal Component Analysis (PCA) indicating eigenvalues and explanatory values for all axes. The axis highlighted in bold represents the VEGETATION parameter.

	Eigenvalue	Difference	Proportion of variance explained	Cumulative value
PC 1	**1.899021**	**0.84203244**	**0.2713**	**0.2713**
PC 2	1.056989	0.1409046	0.151	0.4223
PC 3	0.916084	0.02300987	0.1309	0.5532
PC 4	0.893074	0.05103308	0.1276	0.6807
PC 5	0.842041	0.05173223	0.1203	0.801
PC 6	0.790309	0.18782675	0.1129	0.9139
PC 7	0.602482		0.0861	1

Appendix 3.B. Results of the PCA-analysis indicating eigenvectors for all Principal Components (PCs). The axis highlighted in bold represents the VEGETATION parameter.

	PC1	PC2	PC3	PC4	PC5	PC6	PC7
Nest concealment north	**0.438161**	0.449034	0.263218	0.262366	-0.1491	-0.22962	-0.62714
Nest concealment east	**0.413222**	0.482637	-0.30639	0.311656	-0.12273	0.218739	0.585148
Nest concealment south	**0.376372**	-0.118744	-0.57879	-0.12786	0.636382	0.057583	-0.29086
Nest concealment west	**0.424838**	-0.023693	0.324629	-0.46804	0.172493	-0.56246	0.385223
Nest concealment above	**0.263501**	-0.518513	0.395973	0.627598	0.285482	0.106894	0.134582
Nest concealment below	**0.396838**	-0.130991	0.271681	-0.44923	-0.21084	0.70404	-0.09809
Percentage of canopy cover	**-0.297**	0.514543	0.407253	-0.05428	0.634049	0.269549	0.0597

Appendix 3.C. Parameter estimates for the most parsimonious logistic-exposure model (based on AICc values, see Table 3.3).

Parameter	Df	Estimate	SE	lower 95% CI	upper 95% CI	Chi²	*P*
Intercept	1	3.1436	0.5217	2.121	4.1661	36.31	<0.0001
CLUTCH INITIATION DATE (CID)	1	0.0008	0.0056	-0.0102	0.0118	0.02	0.8879
SEASON 2007/2008	1	-1.9199	0.7099	-3.3113	-0.5285	7.31	0.0068
SEASON 2008/2009	1	-1.1583	0.6262	-2.3857	0.069	3.42	0.0643
SEASON 2009/2010	0	0	0	0	0		
VEGETATION	1	0.1317	0.0838	-0.0325	0.2959	2.47	0.116
DISTANCE TO FOREST EDGE (DIST)	1	-0.0023	0.0024	-0.0069	0.0024	0.92	0.3373
FRAGMENT Chawia	1	1.6685	0.4509	0.7847	2.5522	13.69	0.0002
FRAGMENT Ngangao	0	0	0	0	0		
DIST * FRAGMENT Chawia	1	-0.0106	0.0056	-0.0215	0.0004	3.59	0.0581
DIST * FRAGMENT Ngangao	0	0	0	0	0		
VEGETATION * FRAGMENT Chawia	1	-0.4375	0.1595	-0.7502	-0.1249	7.52	0.0061
VEGETATION * FRAGMENT Ngangao	0	0	0	0	0		
CID * SEASON 2007/2008	1	0.0221	0.0092	0.0041	0.0402	5.78	0.0162
CID * SEASON 2008/2009	1	0.0161	0.0092	-0.002	0.0342	3.04	0.0812
CID * SEASON 2009/2010	0	0	0	0	0		

A female cabanis's greenbul (*Phyllastrephus cabanisi*) feeding her young. © Tom Callens

Food provisioning by helpers lightens the load for breeding females in the cabanis's greenbul (*Phyllastrephus cabanisi*)

Tom Callens, Liesbeth De Neve, Sirke Piirainen and Luc Lens

ABSTRACT

In cooperative breeding species, helpers can improve the performance of the focal breeding pair by means of additive nest-care or lightening the parents' workload. While such cooperative strategies are considered to be most common in the Afrotropical region and Australasia, the social organization and nesting behavior of many tropical species remains poorly known. We quantified the incidence of helping behavior at nests of the little studied cabanis's greenbul (*Phyllastrephus cabanisi*) over a three-year period, in two indigenous cloud forest fragments in SE Kenya. By assessing helper effects on reproductive parameters and food provisioning rates to the nestlings, we further tested if parents adopt additive or compensatory strategies if assisted by helpers. Video-recordings and observations showed that 38% of all breeding pairs were assisted by one or more helpers, hence qualifying *P. cabanisi* as a cooperative breeder. The presence of helpers did not differ between years or forest fragments, nor did it affect laying date, brood size, overall provisioning rate and fledgling weight. However, helpers significantly decreased the provisioning rate of breeding females across years and fragments. Such a load-lightening strategy might positively affect female fitness in terms of increased breeding attempts, condition and/or survival.

INTRODUCTION

In cooperatively breeding bird species individuals regularly forego the opportunity to breed independently and instead help caring for the offspring of conspecifics (Brown 1987, Cockburn 1998, Arnold and Owens 1998, Koenig and Dickinson 2004). Since prompt dispersal and independent reproduction are deemed as the optimal strategy to maximize lifetime reproductive success, the paradox of cooperative behavior has been intensely debated over the last decades, giving rise to numerous non-exclusive hypotheses (e.g. *ecological constraint* hypothesis (Emlen 1982 a,b), *delayed dispersal* hypothesis (Koenig *et al.* 1992) and *life history* hypothesis (Brown 1987, Arnold and Owens 1998), see reviews and discussions in Cockburn 1998, Hatchwell and Komdeur 2000, Covas and Griesser 2007, West *et al.* 2007, Hatchwell 2009). Measuring the costs and benefits of helping for both the helpers and the breeders is crucial for testing these conceptual models as most of them build on the fundamental assumption that helpers enhance the reproductive success of the breeding pair they assist (Cockburn 1998, Dickinson and Hatchwell 2004, Cockburn *et al.* 2008). Helpers can improve the performance of the focal breeding pair (and potentially obtain indirect fitness benefits) depending on the decision of the breeders on how to use the helper contributions. On the one hand, the focal breeding pair can adopt the strategy of maintaining the same parental effort regardless of helper presence. In this case, helper care is additive and can result in an increase of the number or quality of the offspring produced (Emlen and Wrege 1991, Komdeur 1994, Boland *et al.* 1997, Hatchwell 1999, Woxvold and Magrath 2005). On the other hand, the "load-lightening" hypothesis poses that the focal pair can compensate for the care of the helpers by reducing their own effort and hence the costs of reproduction, but thereby overall care to the brood does not increase and no helper effects on the production of young are found (Crick 1992, Dickinson and Hatchwell 2004). In this case, the breeders can increase their condition, survival, or the number of breeding attempts (Langen and Vehrencamp 1999, Heinsohn 2004, Cockburn *et al.* 2008). Both strategies are not mutually exclusive, but the propensity to one of them seems to depend on whether nestling starvation is frequent (Hatchwell 1999): parents adopted the model of additive care in species where nestling starvation is common while they inclined to adopt load-lightening in species where nestling starvation is rare and additional help has a smaller effect on productivity (Heinsohn 2004 and references therein).

Evaluating the benefits of helping has proved to be complex and problematic because it is often difficult to disentangle helper contributions from territory or breeder quality (Cockburn *et al.* 2008, Lloyd *et al.* 2009). In addition, benefits of helping can be condition dependent and vary from year-to-year, with a stronger effect of helpers under poor *vs* good environmental conditions (Magrath 2001, Blackmore and Heinsohn 2007, Covas *et al.* 2008, but see Baglione *et al.* 2006 and Koenig *et al.* 2011 for favorable conditions promoting delayed dispersal and helping). Furthermore, cooperative breeding could also be favored by selection to cope with unpredictable environmental conditions (Cockburn and Russell 2011, Jetz and Rubenstein 2011). Environmental uncertainty resulting from fragmentation and climatic variation are likely to be important drivers of temporal variation in selection pressures and therefore are expected to impact the evolution of behavioral, morphological and physiological traits, including cooperation (Rubenstein and Lovette 2007, Cockburn and Russell 2011, Jetz and Rubenstein 2011).

Here we report on a three year study of helping behavior during breeding in fragmented populations of the cabanis's greenbul (*Phyllastrephus cabanisi*), an Afrotropical forest species so far considered as non-cooperative (Keith *et al.* 1992, Cockburn 2006, Jetz and Rubenstein 2011). Cooperative breeding is globally considered to be most common in the Afrotropical region and Australasia (Arnold and Owens 1998, Jetz and Rubenstein 2011), but concurrently the social organization and nesting behavior of many tropical species remains completely unstudied (Cockburn 2006, Smith *et al.* 2008, Cockburn and Russell 2011). This lack of knowledge can strongly hinder comparative studies using the distribution of cooperative breeding species to resolve the challenging quest for general selective pressures underlying cooperative breeding (Hatchwell 2009, Cockburn and Russell 2011, Jetz and Rubenstein 2011). *P. cabanisi* populations were studied in the Taita Hills (Kenya), an isolated forest archipelago that has been severely fragmented into smaller forest blocks, embedded in a densely populated mosaic (Pellikka *et al.* 2009). Forest fragments differ largely in size and the degree of disturbance (Beentje 1987, Wilder *et al.* 1998, Chege and Bytebier 2005, Pellikka *et al.* 2009) and may therefore affect ecological conditions of the inhabiting bird populations (Lens *et al.* 1999, Lens *et al.* 2002). We examined differences in reproductive performance of *P. cabanisi* and the incidence of cooperative groups over a three-year period in two forest fragments that differ in their degree of degradation. Furthermore, we tested if parents adopt additive or compensatory strategies if assisted by helpers, by examining the effect of the presence of helpers on reproductive parameters and provisioning rates.

MATERIAL AND METHODS

Study area

The study was performed during three consecutive years (2007-2010) in the Taita Hills (SE Kenya, 03°24'S, 38°21'E). These mountains represent the northernmost part of the Eastern Arc Mountains biodiversity hotspot of Kenya and Tanzania (Lovett and Wasser 1993, Mittermeier *et al.* 2005) and have been classified as an Important Bird Area (Bennun and Njoroge 1999). Between 1955 and 2004, the total area of indigenous forest cover decreased by ca. 50% (Pellikka *et al.* 2009), mainly due to clearance for small-subsistence agriculture (Bennun and Njoroge 1999, Myers *et al.* 2000). At present the Taita forest archipelago covers an area of 430 ha of indigenous forest fragments isolated from other highlands by over 80 km of semi-arid plains in either direction (Lovett 1985, Pellikka *et al.* 2009). Nest data were collected in two of the larger fragments, i.e. Ngangao forest (NG, 120 ha) and Chawia forest (CH, 86 ha) (Fig. 2 General Introduction), whereby CH is much more degraded compared to NG due to a long history of disturbance in the former fragment (Beentje 1987, Wilder *et al.* 1998, Chege and Bytebier 2005, Pellikka *et al.* 2009).

Study species

P. cabanisi is a medium-sized, insectivorous specialist of central- to east-African (sub)tropical moist forest (Keith *et al.* 1992, Bennun *et al.* 1996). Globally, the IUCN conservation status of the species is currently evaluated as 'Least Concern' (Birdlife International 2012). Compared to six sympatric forest birds, the species is moderately sensitive to indigenous forest disturbance (Lens *et al.* 1999) with restricted dispersal between isolated forest fragments (Lens *et al.* 2002) and genetic indications of moderate historical loss of mobility (Callens *et al.* 2011). *P. cabanisi* constructs an open cup-shaped nest, usually not higher than 1.5 m above the forest floor, and generally lays a clutch of 2 (range 1-3) eggs (Keith *et al.* 1992) which are incubated by both sexes (mainly ♀) during 17 days (Chapter 3). Nestlings subsequently need a period of 11-13 days to fledge (Chapter 3). Fledged young stay with their parents during the post-fledgling period, even up to the next season (Keith *et al.* 1992). Besides insects, *P. cabanisi* occasionally feeds on fruits (Keith *et al.* 1992, Githiru *et al.* 2005) but supplies nestlings with invertebrates (Keith *et al.* 1992) and even small vertebrates such as little tree frogs and lizards (*pers. obs.*).

Field data collection

Intensive ringing bouts have been carried out in the area since 1996, and by today about 75% of the population of *P. cabanisi* in the Taita Hills is ringed with an aluminum ring in combination with a unique set of three color rings. Nests were intensively searched for throughout all three breeding seasons. Once a nest with eggs or nestlings was detected, we recorded brood size and performed a visual identification of breeding parents and helpers. Almost 60% of the nests were detected during the incubation or nestling stage, and could only be revisited every third or fourth day to minimize disturbance. Therefore, the exact age of the nestlings was determined based on the developmental stage of the feathers. Digital pictures were taken at each nest visit and these pictures were compared to a picture set consisting of daily pictures taken from hatching to fledging from nestlings with known age (see Appendix 5.A). In that way, we could determine the exact age of the nestlings, and also derive the hatching date (cf. clutch initiation date, for details see Chapter 3).

Video-recordings

To monitor parental behavior, nests were videotaped continuously during the morning (7:00-12:00) or afternoon (12:30-18:00) using a Sony HDR-SR7E High Definition video camera. Efforts were made to videotape each nest with the nestlings at an age of 6 days (range 3-10 days, mean = 6.4 ± 1.3 days). The video camera was positioned at a distance of 2-5 meters from the nest, protected from dust and moisture by a waterproof sports container (filled with silica gel sachets), mounted on a tripod and camouflaged with an army color patterned poncho to limit visual disturbance (see Photo 4.1). Videos were viewed with the Program Vegas Movie Studio Platinum version 8.0 to extract the following data of interest during standardized time frames (7:00 – 11:00 or 13:00 – 17:00): time of arrival of a bird at the nest, color band combination (if the bird was ringed) and time of departure. From these data the following variables were derived: (i) ‘presence of helpers’ (binomial): ‘0’ if no helping behavior was detected, ‘1’ if we detected other birds at the nest than the breeding couple; (ii) ‘number of helpers’: number of birds aiding in food provisioning; (iii) ‘overall nest provisioning rate’: number of feedings per hour delivered at a nest; (iv) ‘provisioning rate by the breeding female’: number of feedings per hour by the ‘main’ adult breeding female.

Photo 4.1. Camera setup at a *P. cabanisi* nest. The red circle indicates the nest location.

We detected a total of 223 active nests over the 3 breeding seasons, but due to predation events during incubation and the early nestling period, we only could successfully videotape 103 nests, containing 164 nestlings in total (N = 27, 39 and 37 nests in 2007, 2008 and 2009 respectively). In all videotaped nests we managed to extract total provisioning rates and the presence of helpers. In a subset of nests (N = 31), we were also able to identify the breeding female and accurately determine female provisioning rate. Breeding females were identified based on the analysis of the highly conserved W-chromosome linked CHD-W gene from blood or feather samples collected during ringing bouts (details in Lens *et al.* 1998). Only genetically confirmed females, older than two years and showing the strongest nest care behavior (longest duration at the nest), were considered as breeding females. Since we did not observe a clear difference in nest care behavior between the other individuals at the nest, we were not able to reliably separate breeding from helping in males. Between the videotaping and the ringing of the nestlings 19 of the 103 nests (18.4%) were lost due to predation. At the age of 7-12 days (N = 84 nests and 145 nestlings; mean age = 9.4 ± 1.0 days), the nestlings were banded with a numbered metal band, combined with measurements of body weight (accuracy 0.1g). Furthermore, when a nest was lost due to predation at the egg or nestling stage and when a nest fledged, we searched repeatedly for a new nest from 5 days to 14 days after nest loss. However, we could not evaluate the effect of helpers on the number of breeding attempts, since, renesting attempts after nestling predation only occurred in 5% of all nests, and only 2% of all breeding pairs had a successful second brood in the same year.

Statistical analyses

Nest visitation rates in some species can differ between morning and afternoon (Eggers *et al.* 2005), but we did not find significant differences in feeding frequencies between morning and afternoon sessions (General Lineair Model, all $P > 0.80$). We first examined the effects of fragment, year and the interaction (fixed factors) on the presence of helpers by means of a Generalized Linear Model (GZLM, binomial error term). Subsequently, we tested for the influence of the presence of helpers, in addition to fragment and year effects, on reproductive performance by means of General Linear Models (GLM). Dependent variables were (i) laying date, (ii) brood size, (iii) overall nest provisioning rate and (iv) female provisioning rate whereby year, fragment, presence of helpers and all their interactions were modeled as fixed factors to test if helper effects differed between years or fragments. In the analyses on food provisioning, brood size and nestling age were both included as fixed covariates, as well as their interaction terms with the presence of helpers to test if helper effects on food provisioning varied with brood size and nestling age. Finally, the same explanatory variables were fitted in a general linear mixed model (GLMM) to test for effects on fledgling weight, whereby nest was included as a random factor to account for the non-independence of nestlings from the same nest environment. Furthermore, overall nest provisioning rate was included as an additional fixed covariate in the model on fledgling weight. Model simplification was always adopted using stepwise backward-elimination of non-significant explanatory variables. Fixed effects were tested with F-tests in all models and the Satterthwaite method was applied for estimating the degrees of freedom in GLMMs. All statistical analyses were performed in SAS 9.2 (SAS Institute Inc. 2008).

RESULTS

Presence of helpers

A substantial amount of *P. cabanisi* pairs bred with the assistance of 1 to 3 helpers resulting in the following total numbers: in CH: 38% with one helper, 8% with two helpers, and 2% with three helpers (N = 48 nests); in NG: 18% with one helper, 9% with two helpers and 2% with three helpers (N = 55 nests). In 4 nests we identified first-year individuals as helpers, with 3 cases of confirmed offspring from the previous breeding season. The percentage of nests aided by helpers did not significantly differ between both fragments (GZLM, $F_{1,99}$ = 2.47; P = 0.12), but significantly differed between years (GZLM, $F_{2,100}$ = 3.89; P = 0.02) with a decreasing trend over the years (Table 4.1). The year effect on the presence of helpers was not significantly different between fragments (GZLM, $F_{2,97}$ = 1.64; P = 0.20).

Table 4.1. Mean values (± SE) of laying date, brood size and percentage of nest with helpers in two different fragments (Chawia, Ngangao) and three consecutive breeding seasons (2007-2008, 2008-2009, 2009-2010).

	Fragment		Breeding season		
	Chawia	Ngangao	2007- 2008	2008-2009	2009-2010
laying date	12 Dec ± 3 days	31 Dec ± 3 days	28 Dec ± 4 days	6 Dec ± 3 days	31 Dec ± 4 days
brood size	1.67 ± 0.07	1.49 ± 0.07	1.41 ± 0.10	1.56 ± 0.08	1.76 ± 0.09
% of nests with helpers	47% ± 7%	31% ± 7%	59% ± 9%	36% ± 8%	24% ± 7%

Relationships between helpers and reproductive parameters

'Laying date' did not differ significantly between presence or absence of helpers (GLM, $_{1,98}$= 0.02; P = 0.90), but it differed significantly between years (GLM, $F_{2,99}$= 15.74; P < 0.001) and fragments (GLM, $F_{1,99}$= 20.15; P < 0.001). *P. cabanisi* started to breed earlier in the second compared to the first and third study year (Table 4.1). In CH the birds started to breed about 3 weeks earlier than in NG (Table 4.1). All interaction terms were non-significant (all P > 0.23). 'Brood size' did not significantly differ between presence or absence of helpers (GLM, $F_{1,98}$ = 0.77; P = 0.38) nor between fragments (GLM, $F_{1,99}$ = 3.13; P = 0.08), but it did differ between years (GLM, $F_{2,100}$ = 3.63; P = 0.03; Table 4.1). All interaction terms were non-significant (all P > 0.17). During the whole study, we did not detect brood reduction due to starvation at any nest. Fledgling weight did not significantly differ between presence or absence of helpers (GLMM, $F_{1,97}$ = 0.00; P = 0.96) nor did any of the other explanatory

variables had an effect (all $P > 0.09$). Only the age of fledglings remained in the model showing a positive correlation with body mass (GLMM, $F_{1,81.7} = 37.84$; $P < 0.0001$).

Relationships between helpers and provisioning rates

Overall provisioning rate did not significantly differ between presence or absence of helpers (GLM, $F_{1,98} = 1.22$; $P = 0.27$; Fig. 4.1). Overall nest provisioning rate significantly increased with brood size (GLM, $F_{1,99} = 16.45$; $P < 0.0001$) and nestling age ($F_{1,99} = 13.31$; $P = 0.0004$), but we detected no fragment or year difference (all $P > 0.10$). However, the provisioning rate of the breeding female significantly decreased with presence of helpers (GLM, $F_{1,27} = 6.35$; $P = 0.02$; Fig. 4.1), while no year or fragment effects were found (all $P > 0.68$). Also, brood size and nestling age were positively correlated with female provisioning rate (both $P < 0.001$). All interaction terms in the provisioning rate analyses were non-significant (all $P > 0.09$).

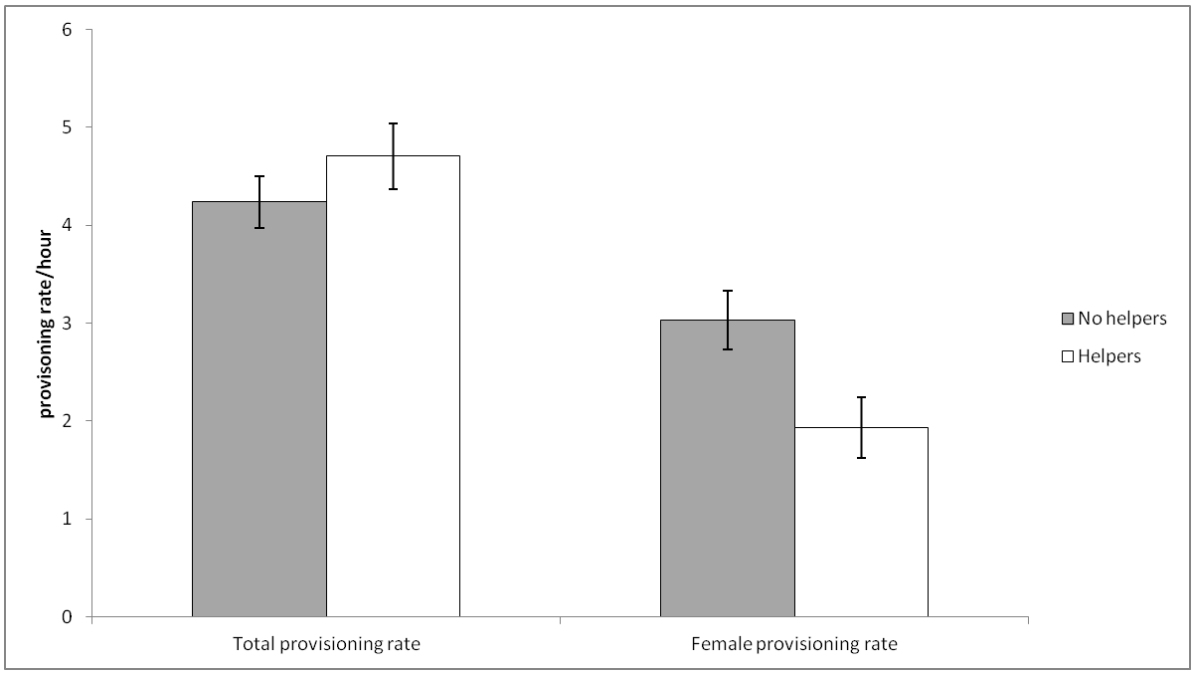

Figure 4.1. Food provisioning rates at *P. cabanisi* nests (mean value ± SE) of all birds (total) and breeding female only (female) with or without the assistance of helpers.

DISCUSSION

We have shown that a substantial amount (38 ± 7 %, N = 3 years) of the detected nests of *P. cabanisi* were attended by more than two birds. Additionally, we found that this newly established cooperative breeding species most likely adopts the load-lightening strategy when assisted by helpers.

In contrast to previous knowledge on *P. cabanisi (*Keith *et al.* 1992, see appendix in Cockburn 2006, Jetz and Rubenstein 2011), we revealed a considerable amount of breeding pairs assisted by helpers (in some years up to 60%; Table 4.1). In accordance with the definition used by Cockburn (2006) considering species with more than 10% of the nests with helpers as cooperative breeders, our data do characterize *P. cabanisi* as a cooperative breeder. *P. cabanisi* was known before to delay dispersal and to form family groups during the non-breeding season with fledged young remaining with their parents, even up to the next season (Keith *et al.* 1992). In the majority of cooperatively breeding species, delayed dispersal is a prerequisite for helping behavior to develop (Hatchwell and Komdeur 2000). Ecological constraints, such as high dispersal costs, shortage of territory vacancies or breeding partners have all been identified as important variables limiting the possibility of independent breeding and promoting cooperative breeding. Both observational and experimental studies have provided consistently strong evidence that specific ecological and/or demographic constraints can successfully explain variation in the occurrence of helping behavior among populations of the same species (Hatchwell and Komdeur 2000, Koenig *et al.* 2011). Given the highly fragmented habitat of *P. cabanisi* in the Taita Hills and the fact that the between-fragment matrix poses substantial limits to landscape-level dispersal in this species (Callens *et al.* 2011), helping behavior could be promoted in this population due to high dispersal costs. Also, large populations of cooperative breeders are hypothesized to be less vulnerable to demographic and environmental stochasticity because helpers provide a pool of replacement breeders and buffer the effects of mortality and productivity on the breeder population size (Walters *et al.* 2004). Future studies might provide answers to these interesting questions.

With respect to provisioning rates, we found that nestlings of larger broods received more feeding visits, possibly explaining why nestling starvation was very rare, and that there was no effect of brood size on fledgling weight. We did not detect positive effects of the presence of helpers on brood size, overall provisioning rates, or nestling weight. Thus, there was no

evidence that helpers increased the reproductive output and thereby had an additive value (Hatchwell 1999). Besides, given that nestling starvation is uncommon in this species, additional care is less likely to strongly influence offspring fitness (Heinsohn 2004). Instead of an increased reproductive output in the presence of helpers, we found support for the load-lightening hypothesis (Crick 1992, Hatchwell 1999). Breeding females maintained a lower provisioning rate when assisted by helpers, which did not affect the total provisioning rate to the offspring. This compensatory behavior might have fitness benefits for the breeding female in terms of increased breeding attempts, condition and/or survival (Cockburn *et al.* 2008). However, our results should be interpreted with caution because the effect of helpers may be confounded by the effect of habitat and/or breeder quality (Brown *et al.* 1982, Dickinson and Hatchwell 2004). Disentangling these effects has been problematic in the past, and by today comparing the performance of identical breeding pairs in consecutive years with and without helpers seems the best way for evaluating the benefits of helping (Cockburn *et al.* 2008, Lloyd *et al.* 2009). Unfortunately we do not have yet long-term data on the breeding performance and survival of breeding females in this population and also the lack of other indicator variables of reproductive performance, such as fledgling survival, and the identification of the helpers as kin and their sex-ratio, leave many interesting questions open for future studies on this new cooperatively breeding species.

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CONTRIBUTIONS

Intensive nest searching was executed by six experienced local field workers (M. Chovu, L. Chovu, A. Mwakulomba, P. Kafusi, I. Mwashighadi and N. Mkombola). T. Callens supervised these search activities in the field, personally executed all nest checks after initial detection (until predation/fledging event), ringed/measured all nestlings and carried out all camera work. Concerning these activities, T. Callens was temporarily assisted by master students (C. Hallmann, S. Piirainen), a local field assistant (M. Chovu) and a volunteer (A. Callens). Bachelor students S. Dionys, J. Hillaert, D. Van de Loock and master student S. Piirainen watched the videotapes. T. Callens verified these data, performed all statistical analyses and wrote the article. C. Vangestel provided statistical assistance. L. De Neve and L. Lens assisted throughout the writing phase.

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APPENDICES

Appendix 5.A. Ageing time series based on pictures of *P. cabanisi* nestlings taken at a specific age.

Day 1

Day 2

Day 3

Day 4

Day 5

Day 6

Appendix 5.A. continued

Day 7

Day 8

Day 9

Day 10

Day 11

Day 12

View from Chawia towards Mbololo isolate. © Tom Callens

Fine-grained spatial genetic structure in a cooperatively breeding bird of fragmented Afrotropical cloud forests

Tom Callens, Carl Vangestel and Luc Lens

ABSTRACT

In cooperatively breeding species, philopatric offspring can lead to a strong clustering of closely-related individuals, thereby increasing the potential of inbreeding costs. Here we analyzed variation in ten polymorphic microsatellite DNA loci to examine fine-grained patterns of genetic structuring and relatedness within and among fragmented populations of the cabanis's greenbul (*Phyllastrephus cabanisi*), a cooperative breeder of the Taita Hills cloud forests. Local auto-correlation analysis provided evidence of positive genetic structure within 300 m distance ranges, which is consistent with behavioral observations of short-distance natal dispersal. At a landscape scale, individual-based auto-correlation values decreased over time, supporting earlier results on increased gene flow over the past decade. Finally, Bayesian mixture analysis of kinship distributions revealed higher proportions of close kin in the smallest forest fragments. Results of this study confirm that individual-based genetic analyses at multiple spatial scales can provide a powerful alternative to population-level analyses. However, interpretation of patterns of genetic relatedness strongly hinges on appropriate sampling regimes, in particular when sampling individuals from numerous discrete biological units, such as cooperative breeding flocks, over large geographic areas, as this may artificially lower levels of average relatedness under strong local genetic similarity.

INTRODUCTION

The escalating rate at which tropical rainforest is fragmented and degraded (Laurance 1999, Brooks *et al.* 2002) imposes strong threats to the long-term persistence of rainforest specialist species (Sodhi *et al.* 2006, Ferraz *et al.* 2007, Sodhi and Smith 2007). Population genetic theory predicts that loss of genetic diversity due to small effective population sizes and low between-fragment gene flow will ultimately decrease the long-term viability of fragmented populations (Frankham *et al.* 2002, O'Grady *et al.* 2006). Tiny and highly-isolated populations in particular are prone to inbreeding depression through incestuous mating, leading to loss of heterozygosity and accumulation of mildly deleterious alleles (Frankham 1995, Higgins and Lynch 2001, Frankham *et al.* 2002, Keller and Waller 2002, Mendez *et al.* 2011).

Species with complex breeding systems, such as cooperative breeders, are considered to be more sensitive to habitat fragmentation compared to pair-forming species, especially when populations become small and isolated (Walters *et al.* 2004, Fischer and Lindenmayer 2007). Many of these species show reduced post-natal dispersal, with offspring helping their parents to raise subsequent broods (Cockburn 1998, Ekman *et al.* 2004). Several studies have detected positive local genetic structure with above-average levels of genetic relatedness in cooperative breeders (Double *et al.* 2005, Temple *et al.* 2006, Woxvold *et al.* 2006, Beck *et al.* 2008, but see Blackmore *et al.* 2011 for absence of local scale genetic structure). Such aggregation of closely-related individuals may result in higher incidence of incestuous mating, especially if habitat fragmentation does beget population fragmentation (Koenig and Haydock 2004).

Studies of genetic structure and gene flow are traditionally performed at the level of "populations", whereby equilibrium-based statistics such as Wright's (1931) F-statistics are applied to assess the degree of genetic differentiation among them. Such methods, however, are often based on inappropriate assumptions (see Whitlock and McCauley 1999 for a review), while *a priori* designation of population units may often lead to doubtful estimates of genetic structure (Pritchard *et al.* 2000, Rueness *et al.* 2003, Mank and Avise 2004). Most importantly, use of these statistics does not allow to quantify "within-population" levels of gene flow without invoking artificial substructuring (Manel *et al.* 2003). Alongside methodological improvements on the analysis of F-based statistics, statistical models have been developed to quantify genetic structuring by assigning individuals to their most likely

population of origin, with operational units being "individual genotypes" rather than arbitrarily-defined population entities (Corander *et al.* 2004, Mank and Avise 2004, Durand *et al.* 2009, François *et al.* 2006, 2010). Despite the irrefutable advantages of such approach, Bayesian clustering analyses do not provide information on local within-population genetic structure either.

Here we apply an individual-based statistical method that is not biased by defining appropriate population boundaries, termed *spatial auto-correlation analysis* (Peakall *et al.* 2003, Double *et al.* 2005), to study fine-grained patterns of spatial genetic structure in a cooperatively breeding forest bird. Despite the fact that this method earlier revealed fine-grained genetic patterning in a variety of species (e.g. beetles: Schmuki *et al.* 2006; birds: Temple *et al.* 2006, Beck *et al.* 2008, Wilson *et al.* 2011, Mendez *et al.* 2011; bush rats: Peakall *et al.* 2003; frogs: Johansson *et al.* 2005; geckos: Hoehn *et al.* 2007), it is still rarely applied in a conservation context (Blouin 2003; but see Vangestel *et al.* 2011 for a recent example). Cabanis's greenbul *(Phyllastrephus cabanisi*) is a common forest-interior species of fragmented cloud forest in south-east Kenya that was recently established as a facultative cooperative breeder (Chapter 4). While such breeding may potentially generate non-random genetic patterns at small spatial scales, earlier genetic studies on this species were performed at population-level only (Chapters 1 and 2). Using a set of ten polymorphic microsatellite DNA loci, we here study individual-based genetic distance measures at different spatial scales, addressing the following questions: (i) do genetic and geographical distance matrices covary at a local scale (i.e. within fragments)?; (ii) do genetic and geographical distances covary at a landscape level (i.e. among fragments) and do these associations change in strength over time?; (iii) is there evidence for spatial and temporal heterogeneity in the distribution of relatedness estimates?

MATERIAL AND METHODS

Study area and species

The Taita Hills (SE Kenya, 03°24'S, 38°21'E) represent the northernmost extreme of the Eastern Arc Mountains, a chain of mountains that run from south-eastern Kenya to southern Tanzania (Lovett and Wasser 1993) and boast a high diversity of flora and fauna, high levels of endemism, but also faced with high levels of threat (Mittermeier *et al.* 1998, Newmark 1998, Stattersfield *et al.* 1998). Forest loss within the Eastern Arc Mountains has been both rapid and drastic and indigenous forests are currently scattered over ca. 5076 km² (Platts *et al.* 2010). Within the Taita Hills, the total area of indigenous forest cover decreased by ca. 50% between 1955 and 2004 (Pellikka *et al.* 2009), mainly due to clearance for small-subsistence agriculture (Bennun and Njoroge 1999, Myers *et al.* 2000). At present, the Taita forest archipelago covers 430 ha of indigenous forest fragmented into three larger patches (between 86 and 185 ha) and eight tiny remnants (2-8 ha), located on two mountain isolates (Dabida and Mbololo) separated by a low-altitude valley (Paranga) (Beentje 1987, Adriaensen *et al.* 2006, Pellikka *et al.* 2009, Fig. 2 General Introduction).

P. cabanisi is a medium-sized passerine that inhabits central- to east-African moist forest (Keith *et al.* 1992, Bennun *et al.* 1996) and displays facultative cooperative breeding behavior, with individuals living in small family groups that consist of the adult breeding pair and up to three helpers (Chapter 4). Globally, the IUCN conservation status of the species is currently evaluated as 'Least Concern' (Birdlife International 2012). Within the Taita forest archipelago, the species has been recorded in all eleven indigenous fragments, but only breeding in the three largest fragments (MB 185 ha, NG 120 ha, CH 86 ha) and two small ones (FU 8 ha, ND 4 ha) (Fig. 5.1: fragments NG, CH, FU and ND are located on a single mountain isolate). In this study, sampling was restricted to breeding populations only, whereby the spatial configuration allowed a one-dimensional (linear) sampling design within as well as among fragments. For the within fragment analyses, individuals were extensively sampled along a 3 km linear transect in fragment MB (see Fig. 5.1A). For the among fragment analyses, individuals were sampled in fragments NG, FU, ND and CH that are positioned along a 13.5 km linear transect in the agricultural landscape matrix (see Fig. 5.1B).

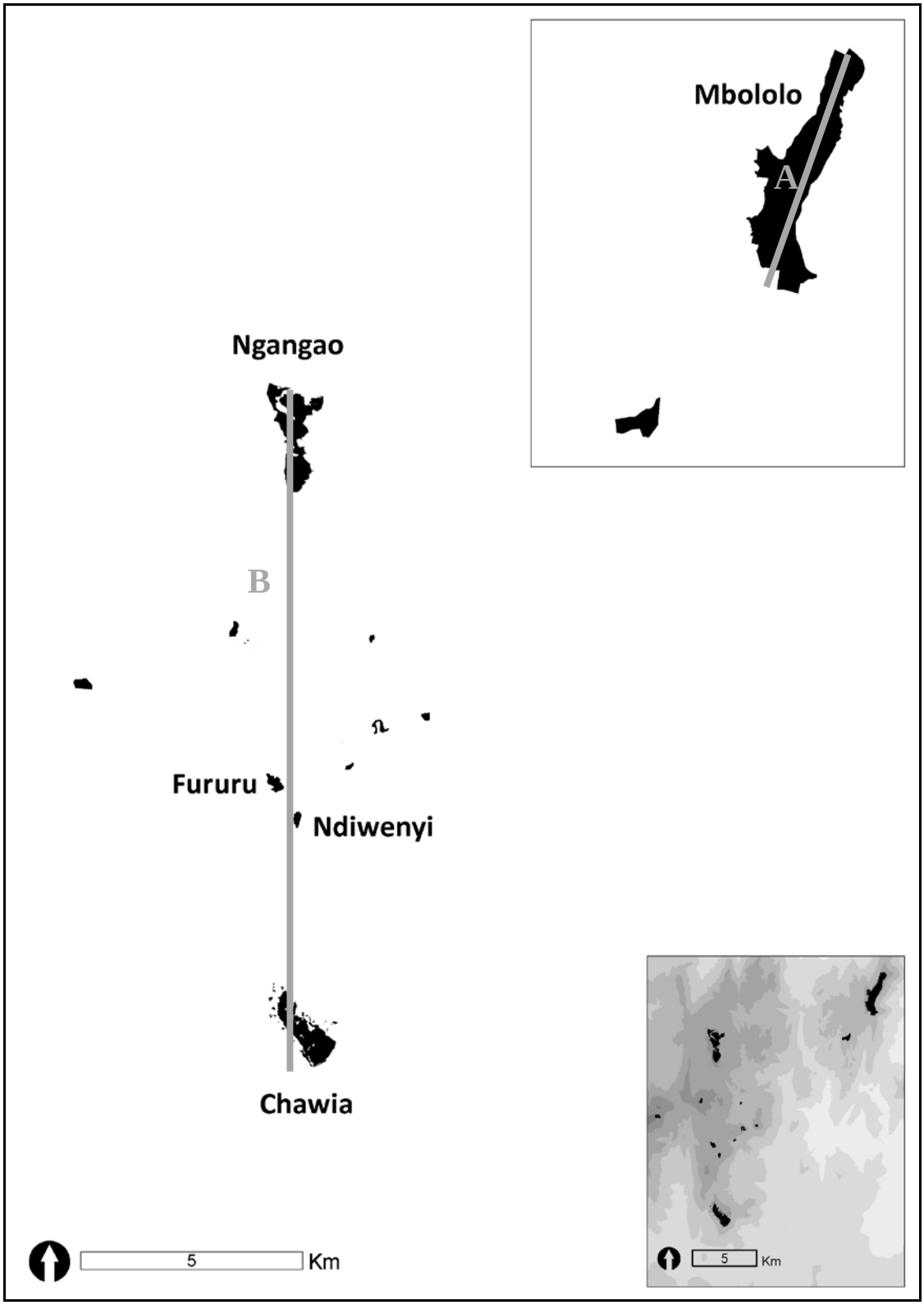

Figure 5.1. Map of the study area with the location of three large (MB, CH, NG) and two small (FU, ND) indigenous forest fragments that hold breeding populations of cabanis's greenbul *(Phyllastrephus cabanisi)*. Auto-correlation analysis was performed along linear transects at two different geographical scales: a local (within-fragment) scale in fragment MB (A), and a landscape (among-fragment) scale including fragments CH, NG, FU and ND (B).

Post-fledging dispersal

Between 1996 and 2010, a total of 2285 individuals were trapped, aged (following Jackson 2005), marked, measured, sampled and released at the original site of capture in fragments MB, NG, CH, FU and ND (see an example of a first-year juvenile in Photo 5.1). Mist-net lines were operated in one to seven 4-ha plots per fragment (proportional to fragment size) and were evenly spaced out in order to sample entire plots. Net positions, net lengths (120 m/plot) and daily trapping efforts (06-18h) were kept constant among trapping sessions. As dispersal distances based on adult capture-recaptures may be biased if fledglings emigrate from their natal territory before first capture (e.g. Van Treuren *et al.* 1999), post-fledging dispersal distances of individuals ringed as nestling were quantified by calculating Euclidian distances between the locations of their nest and the furthest recapture record. Trapping effort was equal in consecutive years, and distances were calculated using Arcmap 9.2 (Environmental Systems Research Institute 1999-2006).

Photo 5.1. Cabanis's greenbul (*Phyllastrephus cabanisi*) first-year juvenile. © Tom Callens

DNA extraction, PCR and genotyping

Upon first capture of an individual, 2-3 µl of blood was collected from the brachial vein and stored in a 95% ethanol buffer. DNA was isolated by boiling in a 5% Chelex solution (Biorad) after an incubation period of 90 min at 55°C in the presence of 100 µg proteinase K (Walsh *et al.* 1991). PCR protocols, product size ranges and optimal reaction conditions of ten variable microsatellite markers are summarized in Chapter 2 (Appendix 2.A). PCR products were visualized on an ABI3130 sequencer (Applied Biosystems) and microsatellite lengths

were determined with GENEMAPPER 4.1. Two random subsets of genotypes were drawn that corresponded to individuals captured during one of two equal time periods: first period (1996-2000): MB=62 ind, NG=65 ind, CH=65 ind, FU=14 ind, ND=18 ind; second period (2006-2010): MB=54 ind; NG=54 ind; CH=55 ind; FU=24 ind; ND =16 ind. The age structure of sampled individuals in both periods did not differ, and individuals sampled during the first period and still alive during the second one, were included in the first period only. We used MICROCHECKER 2.2.3 (Van Oosterhout *et al.* 2006) to identify scoring errors that could be attributed to stuttering, differential amplification of size-variant alleles causing large allele drop-out or presence of null alleles. Running 10000 Monte Carlo simulations and calculating 95% confidence did not provide evidence for null alleles in any of the loci under study. Deviations from Hardy–Weinberg equilibrium and linkage disequilibrium per locus were tested with GENEPOP 4.0 (Raymond and Rousset 1995, Rousset 2008).

Auto-correlation analyses

We assessed fine-grained spatial patterns of genetic structure by quantifying the association between matrices of pairwise spatial and genetic distances (Smouse and Peakall 1999, Peakall *et al*. 2003, Vekemans and Hardy 2004) through spatial auto-correlation analysis in GENALEX version 6.41 (Peakall and Smouse 2006). We defined multiple distance size plots to overcome the problem of sampling scheme dependency (Vekeman and Hardy 2004), and distance classes at which auto-correlation coefficients no longer remained significant were considered to approximate the true extent of identifiable genetic structure (Peakall *et al*. 2003). Spatial auto-correlation analyses were performed both at a local scale (second sampling period only; no GPS locations of captures available in fragment MB during the first period) and at a landscape scale (both sampling periods). Both analyses were run for 9999 permutations and 10000 bootstraps.

Kinship analyses

Kinship analyses can be separated into two types of models: those that assign dyads to discrete relationship categories, and those that estimate relatedness between individuals as a continuous measure of genome-wide identity by descent (Weir *et al.* 2006). Up till now, no single best relatedness estimator exists, because the performance of these estimators appears to be context-specific and to rely on the underlying true genetic population structure (Van de Casteele *et al.* 2001, Wang 2011). As several factors (e.g. sample size, number of

polymorphic markers, relatedness among dyads) may interact in diverse and complex ways, Van de Casteele *et al.* (2001) recommended the use of simulated data to evaluate the suitability of each estimator. Thus, following Van de Casteele *et al*. (2001), we performed Monte Carlo simulations using COANCESTRY Version 1.0 (Wang 2011) to calculate correlation coefficients between different estimated and true relatedness values to select the most optimal relatedness estimator. Based on these simulations, we selected the Queller-Goodnight moment estimator (r_{QG}, Queller and Goodnight 1989) that yielded a strong correlation between true and estimated values (r = 0.75, $P < 0.001$). The asymmetric Queller and Goodnight (1989) index between individuals *x* and *y* (using the former as the reference individual) was calculated as $\hat{r}_{QG} = \frac{\sum_l \sum_k (p_y - \bar{p}_{kl})}{\sum_l \sum_k (p_x - \bar{p}_{kl})}$ where $\bar{p}_{kl}$ equals the population allele frequency of allele *k* at locus *l* over all individuals, p_x equals the frequency of allele *k* in the reference individual (1 or 0.5 for homozygotes and heterozygotes, respectively), and p_y equals the frequency of allele *k* in the individual with which it is being compared (1, 0.5 or 0 for homozygotes, heterozygotes or the absence of allele *k*, respectively). When summed over both individuals, this index transforms into a symmetrical one (Queller and Goodnight 1989).

Next, we explored spatial and temporal differences in kin structure by quantifying the relative contribution of unrelated (U), half-sibling (HS), full-sibling (FS) or parent-offspring (PO) types of relatedness for each fragment-by-period combination. To do this, we first simulated 1000 pairs of each relatedness type based on the observed population allele frequencies with COANCESTRY Version 1.0 (Wang 2011), hereafter called the 'simulated distributions'. All simulated distributions reached normality. Secondly, we calculated the frequency distribution of relatedness coefficients between all n(n-1)/2 dyads per population, hereafter called the 'empirical distributions'. Thirdly, per population we plotted the empirical distribution over the simulated ones and estimated the proportion of each type to the empirical distribution using a finite Bayesian mixture analysis (the empirical distribution was modeled as a mix of normal simulated distributions). Proportions were drawn from a uniform Dirichlet prior distribution generating 100000 posterior samples after discarding the initial 50000 (burn-in) samples. Subsequently, proportions of HS, FS and PO types were pooled into a single 'close kin' type and temporal and/or spatial differences in the proportion of 'close kin' were considered significant if the 95% credibility interval did not contain zero. All analyses were performed in WinBUGS Version 1.4 (Lunn *et al*. 2000).

RESULTS

Post-fledgling dispersal

Analysis of maximum Euclidian distances between natal and recapture sites of fledglings during their first year showed that 83% of all individuals were recaptured within 300 m from their natal site, and 100% within 600 m thereof (Fig. 5.2). No single first-year individual dispersed further than 600 m away and there were no records of among-fragment first-year dispersal either. When including retraps of individuals being older than one year (ringing sessions extended up to Dec 2011), 10% (3 out of 36) were recaptured more than 600 m from their natal site, from which two individuals were between-fragment dispersers (> 4.5 km).

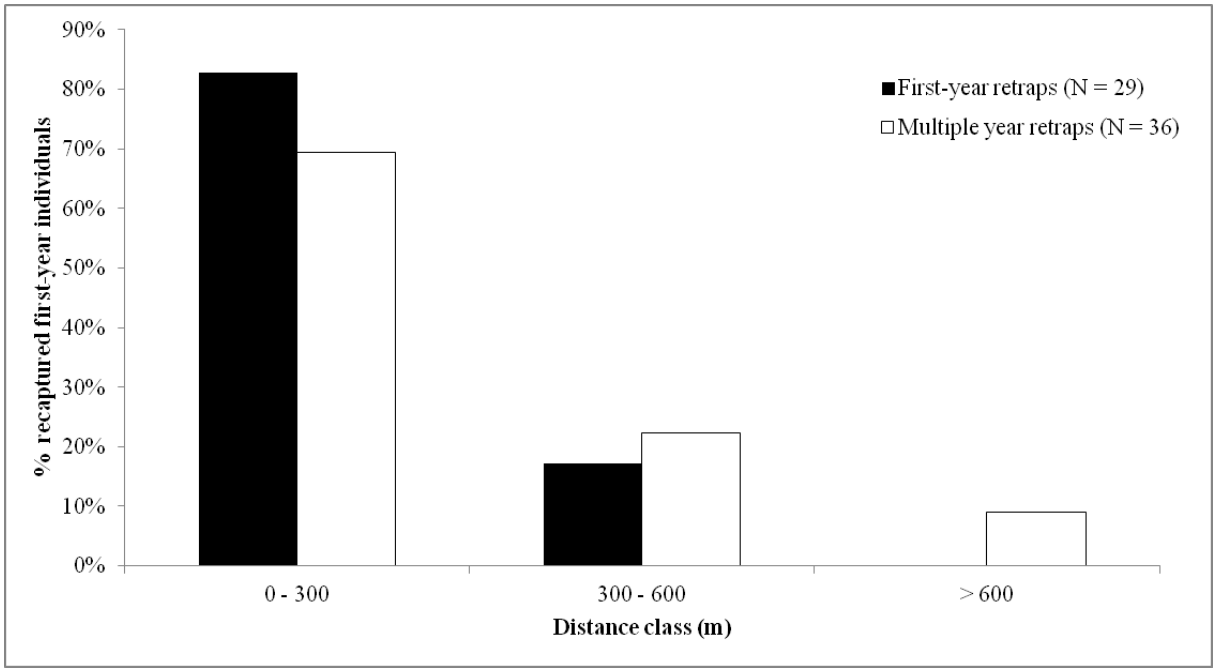

Figure 5.2. Distribution of furthest post-fledging recapture distances of *P. cabanisi* individuals during the first year only or over multiple years.

Microsatellite DNA analysis

There was no evidence for scoring errors due to large allele drop-out or stutter. Loci were highly polymorphic in all populations and consistent with Hardy-Weinberg equilibrium (all $P > 0.05$ after Bonferroni correction; Rice 1989). There was no evidence for linkage disequilibrium after correction for multiple testing (Rice 1989), except for the following combinations: Pca3-WBSW2 (period 1) and Pfi04-Pfl54 (period 2). Since removal of both loci in either period did not affect any conclusion of our study, results presented in this chapter are based on information from all loci.

Spatial variation in genetic similarity

At within-fragment level (population MB), auto-correlogram analysis indicated non-randomly distributed genotypes at small geographical distances, i.e. birds separated by less than 300 m showed strong genetic clustering. Genetic similarity between dyads (pairs of individuals) gradually decreased with increasing geographic distance and auto-correlation coefficients became non-significant at 900-1200 m (Fig. 5.3).

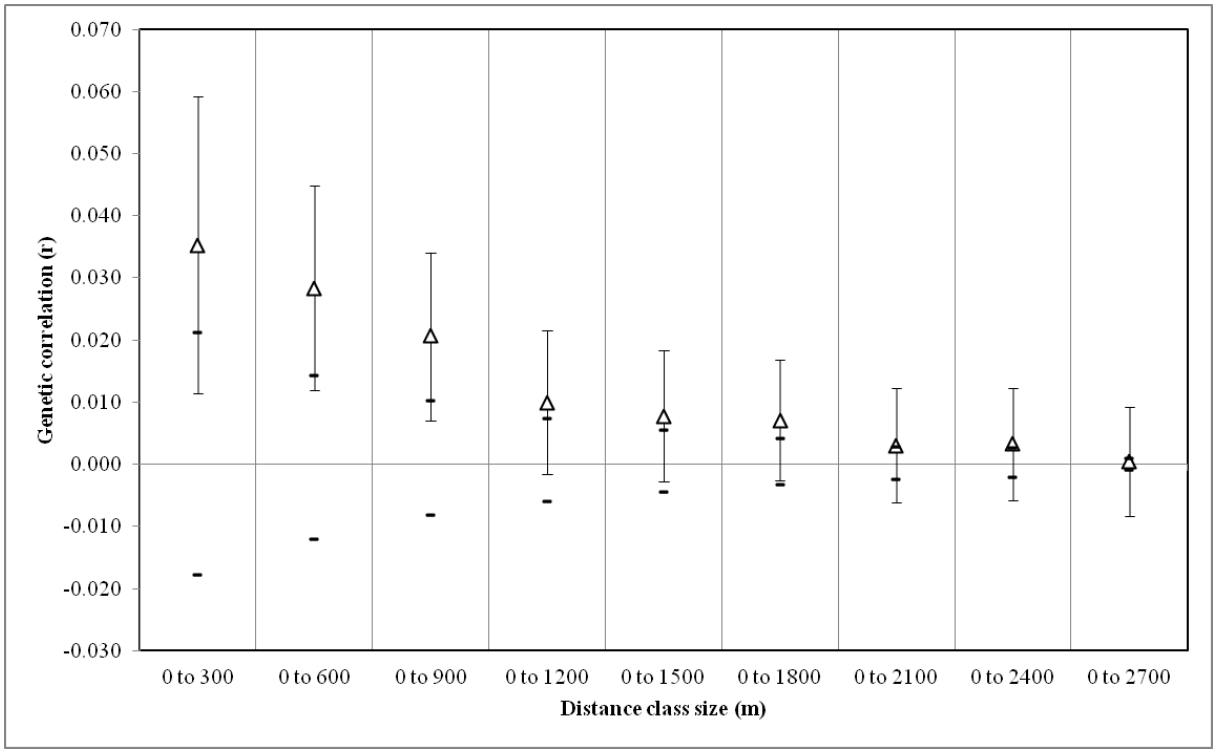

Figure 5.3. Spatial auto-correlogram plots at the scale of fragment MB. The Y-axis depicts genetic correlation coefficients (r) as a function of multiple distance class plots. 95% confidence intervals around null hypothesis (random distribution) are depicted by black marks above/under null value of r. 95% confidence error bars around r were estimated using 10000 bootstraps.

At between-fragment level (populations MB, NG, CH, FU, ND), Bayesian mixture analysis of kinship distributions yielded percentages of close kin ranging between 0.9% (95% CI: 0.2%-1.9%) and 2.5% (95% CI: 1.2%-4.1%) in populations NG, MB and CH (large fragments), and between 7.6% (95% CI: 2.1%-16.1%) and 12.8% (95% CI: 7.5%-19.6%) in populations ND and FU (small fragments), respectively (Fig. 5.4).

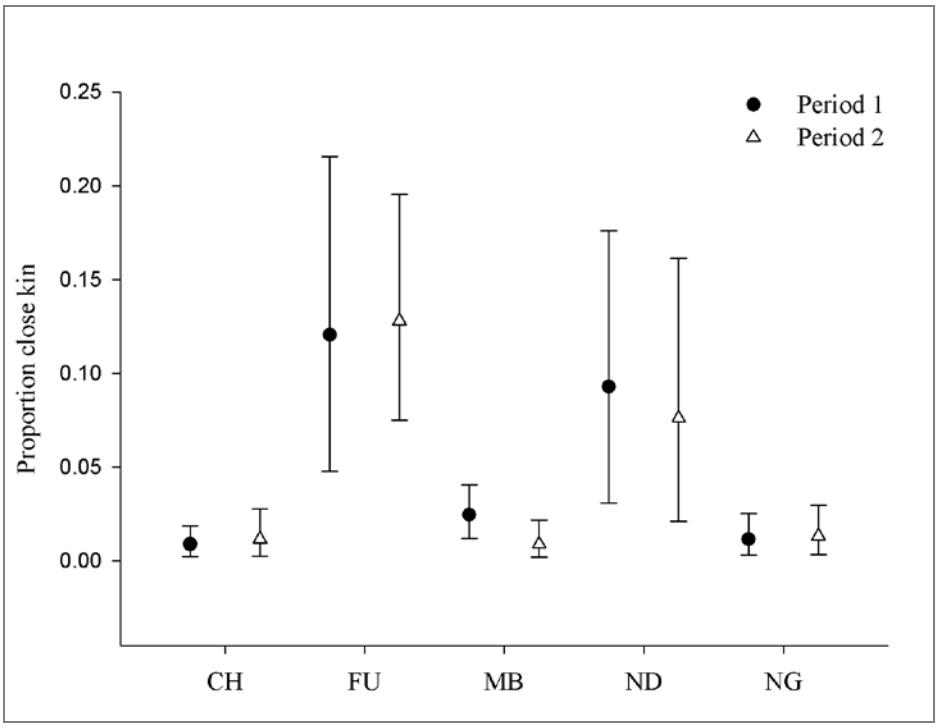

Figure 5.4. Posterior mean proportions of close kin obtained from a finite Bayesian mixture analysis with error bars representing 95% credibility intervals in five indigenous forest fragments during two sampling intervals (period 1:1996-2000; period 2: 2006-2010).

The auto-correlation analysis at landscape scale indicated the strongest genetic affinity among individuals from the within-fragment class (up to a distance of 3000m), gradually decreasing with increasing geographic distance (Fig. 5.5). 95% confidence intervals of auto-correlation coefficients included the null value only if samples from all populations were included.

Temporal variation in genetic similarity

In none of populations NG, CH, FU and ND did the proportion of close kin significantly differ between the first (1996-2000) and second (2006-2010) period (Fig. 5.4). Auto-correlation coefficients were significantly smaller during the second period, but only at larger distances (i.e. > 5000-6000m) (Fig. 5.5).

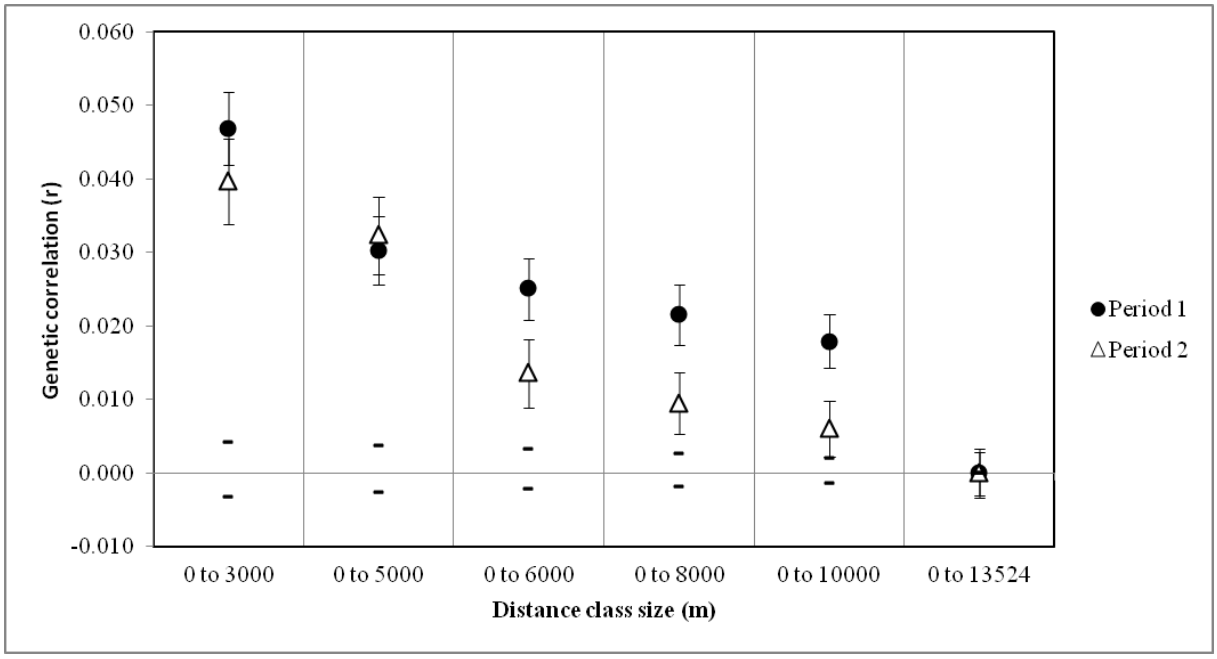

Figure 5.5. Spatial auto-correlogram plots at the scale of the Taita landscape during two time intervals (period 1: 1996-2000; period 2: 2006-2010). The Y-axis depicts genetic correlation coefficients (r) as a function of multiple distance class plots. Class 1 (0-3000 m): within NG, within CH, within FU, within ND, between FU-ND; class 2 (0-5000 m): class 1 + between CH-ND; class 3 (0-6000 m): class 2 + between CH-FU; class 4 (0-8000 m): class 3 + between NG-FU; class 5 (0-10000 m): class 4 + between NG-ND; class 6 (0-13524 m): all pairwise distances. 95% confidence intervals around null hypothesis (random distribution) are depicted by black marks above/under null value of r. 95% confidence error bars around r were estimated using 10000 bootstraps.

DISCUSSION

Auto-correlation analysis revealed fine-grained (i.e. local) spatial genetic clustering in the cooperatively breeding *P. cabanisi*, indicating that individuals were genetically more strongly correlated than expected by chance when separated at distances of 300-900 m only. As no obvious within-forest barriers exist and highly mobile species such as birds should be able to cross such distances without much effort, we believe that the social (breeding) structure of *P. cabanisi*, rather than dispersal limitation per se, is triggering its fine-grained genetic structure. *P. cabanisi* is a group-living species, with adults occupying the same territory for up to 13 years (long term ringing data Taita Hills). Within the study area, the species displays facultative cooperative breeding behavior (Chapter 4) with parents selecting identical nest-sites during consecutive breeding cycles (T. Callens, *pers. obs.*). Albeit based on a small sample, helpers have been identified as territory owners' offspring from the previous breeding cycle (Chapter 4), and all recaptured fledglings settled within 600 m of their natal site during the first year of age. Based on this combined evidence, we suggest that the fine-grained genetic population structure in *P. cabanisi* is likely generated by philopatry and short dispersal distances, a result that is consistent with studies on several other cooperatively breeding species (Double *et al.* 2005, Temple *et al.* 2006, Beck *et al.* 2008).

In addition to local genetic structuring, results of this study also show genetic structuring at a larger (landscape) scale, likely reflecting the combination of high philopatry and low genetic connectivity as a result of strong landscape resistance against dispersal. Such underlying mechanism matches with the lack of evidence for first-year post-fledgling dispersal between fragments (this study) and results from an earlier population-level study that revealed genetic separation among populations of the large fragments (Chapter 2). Here, we further showed high levels of close kin aggregation in both small fragments, possibly reflecting reduced mating opportunities under small effective population sizes (estimated at 4-16 individuals in populations FU and ND, Chapter 2). Overall, however, estimates of relatedness in our study were lower than those reported from other cooperative bird species (e.g. *Struthidea cinerea*; within group relatedness = 0.34-0.36 ± 0.05, Woxvold *et al.* 2006), especially so in the largest fragments. Whilst this may reflect true ecological differences among taxa or regions, under strong local genetic similarity, as was the case in our study, sampling large numbers of individuals from discrete biological units (cooperative breeding flocks) over large

geographical areas (in the larger fragments) may increase the number of unrelated dyads, and hence artificially lower levels of average relatedness (Schwartz and Mckelvey 2009). Hence, between-fragment differences should be interpreted with caution when different local sampling strategies have been applied.

Spatial auto-correlation analysis also showed lower levels of genetic affinity among individuals during the second sampling period, particularly at larger geographical distances (> 5000-6000 m). A decrease in genetic auto-correlation over time may reflect at least two non-exclusive processes, i.e. (i) an increase in genetic drift and/or (ii) a decrease in small-distance gene flow or relative increase in large-distance gene flow (Double *et al.* 2005). An increase in genetic drift during the second period seems unlikely as an earlier population-level study showed effective population sizes to remain equal (or slightly increase) with time (Chapter 2). An increase in between-fragment dispersal is consistent with the observation that at least two small forest remnants were colonized during the last decade (Chapter 2) and the occurrence of occasional long distance dispersal events by older birds (see Fig 5.2; but note the absence of between-fragment dispersal of first year juveniles). A decrease in population differentiation may also result in deflated auto-correlation values (R. Peakall, *pers. comm.*) and such mechanism is consistent with the observed increase in genetic admixture over time (Chapter 2) too. Lack of support for similar temporal effects within the Bayesian analytic framework (i.e. analysis of close kin) may have been caused by the inability to perform this analysis at the appropriate spatial scale. Since auto-correlation coefficients showed strong genetic similarity between dyads at less than 300 m distance, temporal shifts in proportion of close kin should ideally be performed at comparable distances. Small sample sizes at such small spatial scale, however, prevented us from doing so.

In conclusion, this study reveals fine-grained spatial genetic structuring within fragmented *P. cabanisi* populations which remained undetected in earlier population-level studies. Evidence of spatial heterogeneity or temporal shifts in such structure may allow identification of populations of (future) conservation concern. If the higher proportion of close kin in populations from small forest fragments does not reflect a sampling artifact, maintaining and facilitating gene flow within as well as between fragments appears of vital importance to prevent incidences of incestuous mating (Koenig and Haydock 2004) and thus avert future population declines. Yet, both individual-based (this study) and population-level (Chapter 2) analyses suggest increased, rather than decreased, gene flow among fragments. While the

short time frame (i.e. relative to the longevity of tropical birds) of our temporal data hampers firm conclusions on temporal trends, shifts in the genetic population structure of *P. cabanisi* over the last decade at least hint towards population reconnection in the Taita forest archipelago.

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CONTRIBUTIONS

While historical samples (blood and feathers) were obtained from previous research activities (Taita Hills Biodiversity Project), all field data for the contemporary time period were collected by T. Callens, thereby assisted by a ringing team of 2 field assistants: L. Wagura (qualified ringer from the National Museums of Kenya) and L. Chovu (local field worker). DNA-extraction and genotyping were executed by V. Vandomme. All genotyped data were analyzed by T. Callens, except for the kinship analyses which were performed by C. Vangestel. The chapter was written by T. Callens, assisted by L. Lens and C. Vangestel throughout the writing phase.

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Cabanis's greenbul (*Phyllastrephus cabanisi*) at a nest in the Taita Hills. © Alexander Callens

In this concluding chapter, I recapitulate the main conclusions and try to clarify effects of forest loss, fragmentation and degradation on genetic and demographic properties of cabanis's greenbul (*Phyllastrephus cabanisi*) populations by integrating and comparing results of different chapters. Then I consider the cooperative breeding behavior of this species and potential consequences for its long-term viability. Finally, I make suggestions of how findings of this thesis may contribute to conservation management and outline ongoing and possible future research directions that may help clarify some questions that remained unresolved.

OVERVIEW OF THE MAIN RESULTS

Within the isolated Taita Hills of south-east Kenya, severe fragmentation of the original indigenous forest cover resulted in genetic subdivision of *P. cabanisi* populations into three distinct clusters. A comparison of past and current mobility levels of *P. cabanisi* with those of six sympatric forest-dependent species, provided indications for moderate historical loss of mobility over time (**Chapter 1**). In contrast, genetic and demographic spatiotemporal analyses could not substantiate a decrease in between-fragment connectivity over a more recent, fifteen years time span. Rather, contemporary populations showed higher levels of genetic variation and admixture, and effective population sizes remained largely equal or even showed a weak tendency to increase with time (**Chapter 2**). Within fragments, predation levels matched the typical high predation rates on tropical species. However, predation on *P. cabanisi* nests in the Taita Hills was much lower than in similarly-structured forests in a more pristine part of the Eastern Arc Mountains (East Usambara). Predation rates strongly varied in space and time, and a model that combined habitat-, edge- and timing effects was best supported by our data. Nest predation rates increased from the forest edge towards the interior, supporting the notion of an inverse edge effect (**Chapter 3**). Detailed nest observations revealed complex breeding behavior in *P. cabanisi* showing that a considerable amount of breeding pairs were assisted by helpers, characterizing the species as a cooperative breeder. Additionally, we found that breeding females most likely adopt a load-lightening strategy, by reducing provisioning rates

to nestlings when assisted by helpers (**Chapter 4**). While cooperative breeding may potentially generate non-random genetic patterns at small spatial scales, earlier genetic studies on *P. cabanisi* (Chapters 1 and 2) performed at population-level did not capture such fine-grained spatial patterns. Therefore, we studied individual-based genetic distance measures at different spatial scales, which revealed fine-grained spatial patterns of positive local genetic structure, consistent with behavioral observations of short-distance natal dispersal. At a landscape scale, individual-based auto-correlation values also showed restricted dispersal, with the decreasing values over time supporting our other findings of increased gene flow over the past decade (**Chapter 5**).

DOES FOREST FRAGMENTATION LEAD TO POPULATION FRAGMENTATION IN *P. CABANISI?*

Human-induced habitat changes in the Taita Hills, including loss, degradation and fragmentation of indigenous cloud forest since 1955 (Beentje 1987, Newmark 1998, Myers *et al.* 2000, Pellikka *et al.* 2009), have impacted *P. cabanisi* populations in several ways. Earlier research indicated that *P. cabanisi* only occupied four of eight small indigenous forest remnants in the Taita Hills (Lens *et al.* 2002). At community level, variation in patch occupancy was explained by sensitivity to forest degradation, estimated by the level of historical change in developmental homeostasis (Lens *et al.* 1999), and sensitivity to forest isolation, estimated by the level of between-fragment dispersal (Lens *et al.* 2002). When compared to six sympatric bird species, *P. cabanisi* showed intermediate rates in both traits, rendering it a good ecological model species (see General Introduction) to predict population effects of increased habitat fragmentation.

Building on these findings, a comparison of past and current mobility levels as part of this doctoral study provided indications that *P. cabanisi* populations underwent moderate historical loss of mobility (Chapter 1) resulting in genetic population subdivision into three distinct clusters (Chapters 1 and 2). The strong isolation of populations of the Mbololo range from those of the Dabida range (see General Introduction, Fig. 2) probably arose from the presence of a low-altitude valley that reduced gene flow levels even prior to human-induced deforestation. The divergence time between Dabida and Mbololo isolates was estimated at 0.59 million years, based on mitochondrial and nuclear genetic sequence data of chameleons

(Measey and Tolley 2011). Isolation of populations Ngangao (NG) and Chawia (CH) within Dabida most likely originated from increased human population densities and related anthropogenic activities over the last 200 years (Newmark 1998, Pellikka *et al.* 2009, Githiru *et al.* 2011). Deforestation not only caused severe subdivision and isolation of the remnant indigenous forest fragments, but also resulted in a substantial reduction of their average size.

This combination of forest loss, degradation and isolation resulted in low effective population sizes (largest forest fragments: N_e = 55-67; tiny forest patches: N_e = 4-8) that lie well within the range of extinction risk due to endogenous stochastic processes such as demographic stochasticity (e.g. year-to-year variability in reproductive success) and genetic stochasticity (e.g. genetic drift and inbreeding) (Keller and Waller 2002, Frankham *et al.* 2002). Yet, when evaluating shifts in genetic and demographic traits over a recent time span (Chapter 2), we detected relatively high levels of genetic variability (H_E = 0.53-0.63), with no indications for recent bottlenecks and no migration-drift or mutation-drift disequilibria. Likewise, survival rates were not significantly lower in small populations, nor did effective population sizes decrease with time (Chapter 2). Most unexpectedly, genetic connectivity at landscape scale appeared to have increased, rather than decreased, as judged from both genetic [e.g. increased admixture/decreased differentiation (Chapter 2); decreased auto-correlation (Chapter 5)] and demographic estimates [recent dispersal events to previously unoccupied tiny remnants (Chapter 2); occurrence of between-fragment dispersers (Chapter 5)]. While the underlying drivers of this shift in genetic connectivity remain unclear (see following paragraph), results of this study suggest that increased dispersal and gene flow at metapopulation level dampen demographic and genetic effects of forest loss and degradation at the local (population) level, which supports the notion of rescue effects (‘demographic rescue-effect’ *sensu* Brown and Kodric-Brown 1977, Hanski and Gilpin 1991; ‘genetic rescue’ *sensu* Ingvarsson 2001). Additionally, in Chapter 3, we suggested that occurrence of strong variation in responses to differences in nest predation pressure, reflected the ability to cope with complex and variable environmental changes, a process that might be facilitated through maintained genetic variation (Frankham 2005). Even though effective population sizes in our study were well below the generally assumed threshold to maintain evolutionary potential (N_e = 500 to 5000 individuals following Franklin and Frankham 1998, Lynch and Lande 1998), we highlight the importance to maintain or restore gene flow to allow the spread of adaptive complexes between populations (e.g. Templeton *et al.* 2001). However, more research is needed to reveal to what extent strong selective pressures (e.g. variation in predation risk, spatial and temporal

shifts in food availability, dispersal limitations) may influence adaptive evolutionary responses in fragmented populations.

Increased connectivity over the last decade was probably driven by multiple processes. Due to improved protection of the indigenous Forest Reserves and enhanced awareness during the past decade (Githiru *et al.* 2011), within-population expansion in larger or higher quality fragments might have resulted in higher emigration rates in two non-exclusive ways: (i) through higher dispersal propensity under territory saturation versus territory vacancy (Clobert *et al.* 2001, see Chapter 2) as was previously shown for *P. stellata* in fragment NG (Githiru 2003, Githiru and Lens 2006) and/or (ii) through increased group-formation as an adaptive strategy (see next section, '*cooperative breeding: risk or rescue*') generating dispersers in better condition. A recent study of the cooperatively breeding passerine *Turdoides bicolor* in semiarid regions of South-Africa (Ridley *et al.* 2008) showed that group living incurs lower fitness costs compared to 'floating' (i.e. solitary individuals with no fixed territory waiting for territory vacancies or wandering into breeder's territory searching for vacancies; *sensu* Brown 1969). Hence, group living can produce individuals in better condition once they disperse with higher chances of becoming active breeders in new territories (Ridley *et al.* 2008, Tarwater and Brawn 2010), which may lead to increased successful dispersal (Ridley and Raihani 2007). Yet, lack of detailed (telemetric) data on movement behavior prevents us from concluding whether *P. cabanisi* individuals adopt floater strategies next to, or subsequent to, the group-living and helper strategy documented in this study. Regardless of the pre-dispersing strategy adopted by emigrants from high-quality fragments such as NG, the assumption that these fragments acted as the principal source of dispersers to the smaller fragments was supported by the increased census population size without concurrent increase in effective population size in population NG (Chapter 2) in combination with the increased likelihood for individuals from FU (Fururu) and ND (Ndiwenyi) of belonging to the NG cluster (Chapter 2). In turn, due to their geographic positioning in the landscape, the smaller fragments may function as stepping-stones, either within or between generations, for gene flow among the larger populations (Turner and Corlett 1996, Githiru and Lens 2004).

While it is tempting to believe that the overall increase in connectivity may also have been caused by current habitat restoration efforts through matrix enrichment and the conversion of exotic plantations (Githiru *et al.* 2011), such conclusion remains highly speculative given the

short time-frame involved. Moreover, restoration efforts in the landscape matrix may, on the long term, not only directly affect target species, but also have indirect effects through positive effects on the connectivity of predator communities (Fischer and Lindenmayer 2007). Yet, until the identity of the main nest predator(s) of *P. cabanisi* is known, we can only speculate on future effects of matrix enrichment on predator-prey dynamics and population effects (Chalfoun *et al.* 2002).

In addition to the observed increased connectivity, it is important to mention that anthropogenic disturbance might have resulted in a positive effect on the *P. cabanisi* populations through a process of predator-release near forest edges. While nest predation rates are generally elevated in fragmented and detoriated habitat patches (Robinson *et al.* 1995, Lahti 2001), rates in our study area were much lower than those recorded in more pristine forests in other areas of the Eastern Arc Mountains biodiversity hotspot (i.e. East Usambara Mountains; Newmark and Stanley 2011). Landscape modification and human disturbance not only affect the habitat of forest birds but also that of taxa at other trophic levels, such as ground-dwelling or aerial predators (Fischer and Lindenmayer 2007). Although the current understanding of the impact of fragmentation on nest predator communities is limited (Chalfoun *et al.* 2002), some predators might be affected more severely than their prey, leading to changes in predator-prey interactions (Fischer and Lindenmayer 2007). In some tropical landscapes, predators have been or are still severely persecuted (Redford 1992), which may result in decreased predation rates on forests birds and their nests (Stratford and Robinson 2005). In our study area, such process seems most prominent in CH, whereby predators are probably chased into the forest interior, resulting in exceptionally low predation rates along the forest edges of this strongly degraded fragment (Chapter 3). In line with this, predation rates in the largest, most pristine and least populated forest (Mbololo: 90% predated, *unpubl. data*) were comparable to those recorded in continuous indigenous (Afro)tropical forests (Newmark and Stanley 2011). Albeit being a habitat specialist, *P. cabanisi* seems to nest frequently close to the predator-released edges of degraded fragments, which may boost its overall breeding success (Chapter 3). Hence, *P. cabanisi* might temporarily thrive in degraded forest fragments due to a combination of high levels of immigration (Chapter 2), coupled with low levels of nest predation near forest edges (Chapter 3), resulting in higher chances of successful reproduction of residents with immigrants. This may have caused the observed increase in the effective population size of CH over the last decade (Chapter 2).

In conclusion, despite indications of historical mobility loss over time, results on genetic and demographic parameters over a contemporary time frame supported the notion of increased connectivity, most probably originating from increased forest protection and population expansion in the large, high quality fragments, coupled with low predation rates near forest edges in degraded fragments. Hence, our findings are promising in the fact that the detrimental effects of forest fragmentation might be reversible, given that increased dispersal and gene flow at metapopulation level seem to dampen demographic and genetic effects of forest loss and degradation at local population level.

COOPERATIVE BREEDING: RISK OR RESCUE?

For many tropical species, the social breeding system has not been thoroughly studied, and as such, the designation of cooperative behavior is deducted from habits of related species based on molecular phylogeny or systematic resemblance (Cockburn 2006). So far, *P. cabanisi* was categorized as 'non-cooperative' (Cockburn 2006, Jetz and Rubenstein 2011). Yet, video-observations during three consecutive breeding seasons provided solid evidence for food-provisioning by helpers (Chapter 4). Similar studies might reveal other cooperatively breeding species within the family of *Pycnonotidae* in Africa (currently 50% of all species within this family with breeding status 'unknown', Cockburn 2006), because cooperative breeding appears to occur commonly in species with year-round residency, high survival and low fecundity (Arnold and Owens 1998), traits that are typical for (Afro)tropical passerines (Johnston *et al.* 1997, Stutchbury and Morton 2001).

In many cooperatively breeding species, individuals forego the opportunity to breed independently and instead help caring for the offspring of conspecifics (Brown 1987, Arnold and Owens 1998, Cockburn 1998, Koenig and Dickinson 2004). This study yielded preliminary evidence that some helpers were indeed territory owners' offspring (3 out of 4 helpers identified as first-year individuals were confirmed as offspring from the previous breeding season, Chapter 4) and capture-recapture data revealed that first-year birds remain close to their natal sites during their first year (Chapter 5). Depending on the size of the remaining fragments and the connectivity of the landscape matrix, such high level of philopatry and the presence of non-breeding individuals (helpers) may render a species either more or less sensitive to habitat fragmentation (Walters *et al.* 2004).

In large fragments, cooperative breeders are generally presumed to be more persistent than pair-forming species, due to the fact that breeding vacancies can be occupied more rapidly and fluctuations in breeding population caused by demographic and environmental stochasticity can be dampened (Walters *et al.* 2004). Due to the short duration of this study, and without experiments, long-term demographic effects of cooperative breeding cannot be assessed. Theoretically, however, it can be expected that the load-lightening strategy applied by breeding females (Chapter 5), may increase their per capita survival and/or number of breeding attempts (Heinsohn 2004, Cockburn *et al.* 2008) and possibly increase population growth rates (Low *et al.* 2010). We did not find any significant effects of survival and number of breeding attempts for cooperatively breeding females in our study (Chapter 4), though our sample size was only modest (N = 31). Further research is therefore required to examine whether the particular breeding system of cooperatively breeding species increases or decreases their vulnerability against stochastic processes.

In large fragments, cooperative breeding can also be an adaptive strategy for offspring through which they receive direct benefits by delaying dispersal (Covas and Griesser 2007). These direct benefits can arise from 'parental facilitation' through increased access to resources and protection from competitors and predators (reviewed in Ekman *et al.* 2001) or through 'group augmentation', reducing the risk of predation and increasing foraging (Kokko *et al.* 2001). Therefore, juveniles that delay dispersal and become helpers may be in a better body condition once they disperse at later ages, compared to 'floater' strategies in pair-breeding species (Ridley *et al.* 2008, Tarwater and Brawn 2010). In *P. stellata*, a forest-dependent generalist (Bennun *et al.* 1996), costs of dispersing seem to be relatively low, as the species traverses the matrix regularly and rapidly (based on translocations and capture-recapture data) and forages at forest edges and undergrowth outside the forest (Githiru 2003). Nests are constructed at diverse nesting sites (Githiru *et al.* 2005) and the species breeds in all forest fragments, including highly degraded forest patches as small as 0.2 ha scattered across the landscape (Spanhove *et al.* 2009). Thus, for this species, 'floating' towards small unoccupied patches, seems to be a dispersal-strategy without incurring high costs (Githiru 2003). In contrast, breeding populations of the forest specialist *P. cabanisi* (Bennun *et al.* 1996) only occur in 5 of the 11 remaining fragments, individuals remain in the forest interior and are rarely seen or captured outside indigenous forest boundaries (*pers. obs.*), nest building only occurs at selected nest sites (specific plant species and nest height, see Chapter 3), highlighting the species' lower tolerance against secondary matrix habitat. Costs of floating

might be too high for *P. cabanisi* juveniles, thereby selecting for delayed dispersal and group living as an adaptive strategy (Covas and Griesser 2007, Ridley *et al.* 2008, Tarwater and Brawn 2010).

In contrast, in small and isolated populations, aggregation of close relatives in highly sedentary cooperative breeders (Ekman *et al.* 2004, Dickinson and Hatchwell 2004) might increase the risk of incestuous matings and inbreeding depression (Koenig and Haydock 2004). As mentioned earlier, results of this study revealed strong levels of philopatry in *P. cabanisi*, which probably caused the fine-grained spatial genetic structure described in Chapter 5. Additionally, levels of relatedness were higher in populations of small forest fragments (Chapter 5). If the latter become even more isolated, negative genetic effects of cooperative breeding might outweigh the positive demographic ones. Yet, in cooperatively breeding species, inbreeding generally does not occur more frequently compared to pair-breeding species, because many cooperative breeders show mechanisms of inbreeding avoidance to minimize chances of mating with close relatives (Koenig and Haydock 2004). One of these mechanisms, acting to reduce incestuous matings, is sex-biased dispersal, whereby males are generally the more philopatric sex and females settle away further from their natal territory (see examples in Double *et al.* 2005, Temple *et al.* 2006; reviews in Komdeur 2004, Hatchwell 2009). Preliminary results on *P. cabanisi* based on sex-differences in auto-correlograms (data not shown) provided weak genetic indications for female-biased dispersal, but our current genotyped data set was too small to confirm this effect. In small and isolated fragments, such sex-biased dispersal pattern can produce a shortage of mates, which can place serious restrictions on population reproduction and growth, well before inbreeding depression becomes an issue (Dale 2001, Koenig and Haydock 2004). Yet, in *P. cabanisi*, it seems that although most individuals employ a 'search-and-foray' strategy (*sensu* Brown 1987) by residing at their natal territory, some individuals, mainly at later ages, seem to 'depart-and-search' by performing long-distance movements (Chapter 5). Hence, by switching from a 'search-and-foray' to a 'depart-and-search' strategy (see Woolfenden and Fitzpatrick 1984, Stith *et al.* 1996 for identical processes in *Aphelocoma coerulescens*), these individuals prevent complete isolation of the small populations (Walters *et al.* 2004). Given that the effective and census population sizes of small fragments ND and FU do not show any negative trends (Chapter 2), we assume that the occurrence of between-fragment dispersal events (Chapter 5), the high immigration rates from NG towards the small fragments (Chapter 2) and the overall increased connectivity at metapopulation level (Chapter 2 & 5), seem to

prevent that deleterious processes take place in cooperatively breeding species occupying the smallest and most isolated fragments.

CONSERVATION IMPLICATIONS

Results from this study can help to draw conservation management plans for fragmented populations of endangered species on which detailed nest observations or genetic sampling cannot be conducted for logistic or ethical reasons (Githiru *et al.* 2007). In addition to the arguments based on habitat association and shared threats (see General Introduction), *P. cabanisi* can be considered as a good surrogate model for ecologically similar species of conservation concern, for the following reasons. First, *P. cabanisi* appears moderately sensitive to indigenous forest disturbance (Lens *et al.* 1999) and isolation (Chapter 1). Because of its intermediate sensitivity, it can be expected that further habitat fragmentation or restoration may trigger rapid and measurable genetic and demographic effects in this species, while no such responses may be measurable in sympatric but highly mobile/tolerant (e.g. *Nectarinia olivacea*) or highly sedentary/intolerant (e.g. *Turdus helleri*) species (Lens *et al.* 2002). Second, as tropical species are generally characterized by a different set of life history traits compared to their temperate counterparts (Stutchbury and Morton 2001), such as smaller clutch sizes, low number of renesting attempts and higher survival (Johnston *et al.* 1997, Stutchbury and Morton 2001, Peach *et al.* 2001), they are generally considered as K-selected species, whereby lower fecundity and slower development may increase their competitive ability in stable ecosystems such as tropical rainforests (Dobzhansky 1950, Pianka 1970). *P. cabanisi* can be considered as a good model for such K-selected species, given its small clutch size (2-3 eggs, Chapter 3), long lifespan (oldest individual known to be alive for at least 13 years), high survival (mean yearly survival rates: 0.69-0.76, Chapter 2) and high site fidelity with short post-fledging dispersal distances (Chapter 5). For species with similar life history traits, large intact forests are of vital importance because their persistence is mainly determined by their spatial requirement for primary forest habitat (Van Houtan *et al.* 2007) combined with a moderate tolerance of secondary matrix habitat (Gascon *et al.* 1999). Comparative studies have shown that individual species vary widely in their response to habitat fragmentation and that patterns of patch occupancy are primarily limited by dispersal abilities (Lens *et al.* 2002, Sekercioglu *et al.* 2002, Lees and Peres 2006, Burney and

Brumfield 2009, Chapter 1). Results of this doctoral study hence additionally stress the role of life history traits as a major determinant of a species' sensitivity to fragmentation.

Yet, extrapolation of results obtained from surrogate species to ecologically equivalent species should be carried out with caution, as species that resemble in current mobility and genetic differentiation may strongly differ in population responses to habitat fragmentation (Chapter 1). For example, the open-cup type of arboreal nests and presence of helpers during food provisioning may trigger different predator responses (Söderström *et al.* 1998, Newmark and Stanley 2011) and incur different costs and benefits of dispersal in *P. cabanisi* compared to pair-breeding ground- or hole-nesting species that occupy identical habitats. Along these lines, in the sympatric *P. stellata* that builds closed nests at or near ground level, high disturbance levels in fragment CH resulted in high nest predation rates (Githiru *et al.* 2005), low reproductive rates, and a (pseudo)-sink population of which the long-term survival depends on immigrants from larger fragments (Githiru and Lens 2004, 2006). In contrast, human-induced predator-release may have resulted in low predation rates on *P. cabanisi* nests near forest edges in CH, which may - together with increased immigration - have boosted the effective population size in this degraded fragment.

In addition, we want to highlight that even in species displaying similar nesting behavior, one should be careful with across-species interpretations: Because the off-ground, open-cup shaped nests of the critically endangered *T. helleri* and *A. fuscigularis* nests are ecologically more similar to *P. cabanisi* than to *P. stellata*, they might attract identical predators. As such, one might easily conclude that the suggested predator-release hypothesis for *P. cabanisi* might be of conservation importance for recently executed (*T. helleri*, Lens *et al.* 2009) or future (for *A. fuscigularis*) translocation programs in CH in terms of increased breeding success. However, a similar process as was suggested for *P. cabanisi* will only occur if the critically endangered species construct their nests close to the forest edges, which is generally not the case, as the latter species are strongly confined to the forest interior.

Furthermore, results from this study suggest that complex social relationships such as evident under cooperative breeding can either result in rescue effects or increased risk of extinction, depending on the size and isolation of fragments. On the one hand, large populations could function as a refuge, dampening demographic stochastic effects via the presence of a large amount of non-breeders (Walters *et al.* 2004), or providing a safe environment to produce

dispersers in better condition (Ridley *et al.* 2008). On the other hand, small or isolated populations of cooperative breeders may suffer an increased risk of extinction because of demographic stochasticity, genetic stochasticity or low immigration rates (Gilpin and Soulé 1986, Frankham 2005) or because of the need of large tracts of habitat areas and general reluctance to cross gaps by group-living species (Van Houtan *et al.* 2006). Results of our study suggests two additional risks in small populations: (i) strong natal philopatry resulting in high levels of within-fragment relatedness, increasing the risk of inbreeding, and (ii) inbreeding avoidance mechanisms such as sex-biased dispersal resulting in reduced population reproduction or growth.

Thus, we want to stress the importance of detailed validation before implementing surrogate-based conclusions in conservation planning (Murphy *et al.* 2011), as it seems that ecologically similar species may show largely different population responses depending on their dispersal propensity, nesting behavior and breeding behavior. Taking these cautionary notes into consideration, in general, our findings support the recommendation that conservation of fragmented populations of K-selected Afrotropical passerines requires action both within habitat fragments and at the landscape level (Lens *et al.* 2002). Within fragments, habitat loss and deterioration should be kept at a minimum in order to maintain adequate population growth and emigration to other populations. Across fragments, efforts should be made to maximize landscape connectivity in order to allow birds to move between fragments (see Githiru *et al.* 2011 for ongoing conservation projects; but mind the possible effects on predator-prey dynamics).

ONGOING AND FUTURE RESEARCH

Based solely upon our results, it is delicate to make predictions about future genetic and demographic changes given the complex relationships with habitat extent, habitat quality and landscape connectivity. Yet, as part of the doctoral study of Job Aben (University of Antwerp), our baseline genetic data (i.e. pairwise gene flow estimates, effective population sizes) and demographic data (i.e. survival rates, census population sizes) are currently implemented in spatial explicit population viability models (RAMAS GIS 5.0, Akçakaya 2000) which will be used to predict long term species survival under different scenarios of habitat restoration in the Taita Hills. This modelling approach, partially based on ‘landscape

genetics', will allow us to accurately predict and evaluate future effects of proposed management alternatives on *P. cabanisi* and other sympatric species (Manel *et al.* 2003, Storfer *et al.* 2007).

While this doctoral study provided new insights in genetic and demographic effects of tropical forest fragmentation, new questions have arisen while other could not be properly addressed due to lack of time or appropriate methodology. For instance, although we were able to reveal ecologically-significant patterns in rates of nest predation (e.g. inverse edge effect, Chapter 3), we could not reliably infer the identity of the predators. Yet, improved forest quality may enhance local carrying capacities of some predator species, but not of others, nor do different species respond equally to increased landscape connectivity (Fischer and Lindenmayer 2007). Hence, without proper knowledge of the identity of nest predators, it is difficult to make general predictions on the long-term viability of *P. cabanisi* and related forest birds at metapopulation level (Lahti 2001, Chalfoun *et al.* 2002). For instance, if higher predation rates in the forest interiors would mainly reflect high rodent and other small mammalian densities, (re)introduction of top predators may relieve forest birds from higher predation pressure in the interior (Crooks and Soulé 1999, Maina and Jackson 2003), and thus increase overall reproduction success. On the other hand, if the inversed edge-effect would stem from edge avoidancy of large-bodied nest predators (e.g. raptors and monkeys), restoring connectivity, improving habitat quality and increasing fragment size may promote survival of these predators, and at the same time reduce reproduction of the target species. Hence, an observational study of nest predators, for instance with automatically triggered 24/7 infrared cameras (see Robinson *et al.* 2005 for an example, Richardson *et al.* 2009 for a review) combined with a long-term capture-mark-recapture analysis (demographic trends) and radio-telemetry study of the main predators (ranging behavior and activity patterns) is required to study relationships at (meta)community level.

We also provided the first evidence for cooperative breeding in *P. cabanisi.* Although we revealed a positive effect of cooperative breeding behavior on load-lightening in adult females (Chapter 4), more insights into evolutionary routes to, and possible ecological effects of, such behavior under variable levels of habitat fragmentation might be obtained from experiments and more detailed studies on sex-ratios. In many cooperative birds, offspring of one sex (often the males) tend to remain at the natal territory and become helpers or attempt to breed in the vicinity of the parental territory, whereas females tend to disperse (Komdeur 2004, Hatchwell

2009). Although preliminary results of our study indicated weak tendencies of female-biased dispersal, more individuals should be molecularly sexed to accurately determine the sex structure within cooperatively breeding groups. As fitness consequences of helping behavior (Dickinson and Hatchwell 2004) and delayed dispersal (Ekman *et al.* 2004) are believed to be intertwined and vary with environmental conditions (Covas and Griesser 2007), offspring sex ratios can be expected to become skewed to 'helpers' or 'dispersers' as a result of condition-dependent maternal strategies (Komdeur 2004). Significant sex-ratio skews at the facultative level have been found in several bird species, especially when costs and benefits between the sexes differ depending on maternal and/or environmental condition, which is the case for cooperative breeding species (Komdeur 2004). Joint evolution of dispersal and sex-ratio may then be expected if sexes compete differently for resources and when inbreeding load is high (Leturque and Rousset 2003, Guillaume and Perrin 2006). By combining field experiments (e.g. food provisioning and vocal playback), behavioral observations (video-recordings and radio-telemetry) and non-invasive sampling (physiological, phenotypic and genetic), we could assess to what extent properties of individuals, territories and the landscape interact on maternal sex allocation, offspring condition, dispersal and settlement strategies. Under high environmental quality, we would thereby predict mothers to skew sex-ratio to the 'helper' sex (Komdeur 2004), as expected fitness benefits (improved future breeding success) may outweigh expected fitness costs (intra-specific food competition during non-breeding). If, by means of an experimental treatment (extra food provisioning), sex ratios in poor quality territories would become helper skewed, we would predict parents to drive off offspring, as fitness costs to maintain large non-breeding groups in these territories would be expected to become higher. Parents would also be predicted to vary in their tolerance towards philopatric offspring and non-related immigrants (mostly from the dispersive sex) depending on the optimal sex ratio and group size for a given territory quality (Komdeur 2004, Griesser *et al.* 2008). However, group structure and size in high quality territories from strongly isolated and small fragments may be suboptimal as a result of dispersal constraints or stochastic effects. Hence, such groups would then be predicted to be more indulgent to immigrants in order to avoid costs of inbreeding depression (Koenig and Haydock 2004).

Finally, we provided indirect evidence for moderate historical decrease in mobility (Chapter 1), which appears to be counteracted by a recent increase in genetic connectivity (Chapter 2). Despite several independent lines of evidence from this study for a contemporary increase in connectivity, temporal samples were only separated by a single generation interval. Although

this might be sufficient to monitor trends in gene flow and demographic traits at an ecological timescale, it does not allow inferring evolutionary patterns and processes. Yet, while restricted in sample size, individuals of *P. cabanisi* and most other sympatric bird species were collected prior to the strongest episode of forest fragmentation in the Taita Hills (mainly between 1934-1948), and are currently stored in various museums in Kenya, the UK and the USA (Lens *et al.* 1999). These specimens allow a detailed comparison of pre- and post fragmentation levels of both genetic (e.g. allelic richness, private alleles) and phenotypic traits. In view of the inferred differential loss in mobility over time among the set of sympatric bird species, it would be especially interesting to study potential changes in flight morphology, for instance through a comparative study of skeletal elements in the wing in pre-fragmentation (museum specimens) and post-fragmentation (live capture) samples of species that are presumed to differ in the extent of loss of mobility. Earlier comparative studies of migratory species showed that higher mobility can be associated with longer and more pointed wings (Marchetti *et al.* 1995), while others revealed that predation pressure generally shapes wing morphology towards shorter and rounder wings for increased maneuverability (Alatalo *et al.* 1984, Green *et al.* 2009). An x-ray study on a restricted set of live birds and museum specimens from the Taita Hills is currently underway. Evolutionary changes can be observable in only as few as 25 generations (see review in Stockwell *et al.* 2003) and a recent study on temperate and boreal species highlighted rapid evolution in wing morphology over a time-span as short as 100 years only (Desrochers 2010). Thus, a multi-generational approach might reveal whether or not fragmentation-induced mobility and/or predation pressure changes may result in fast evolutionary responses in tropical forest birds such as *P. cabanisi*.

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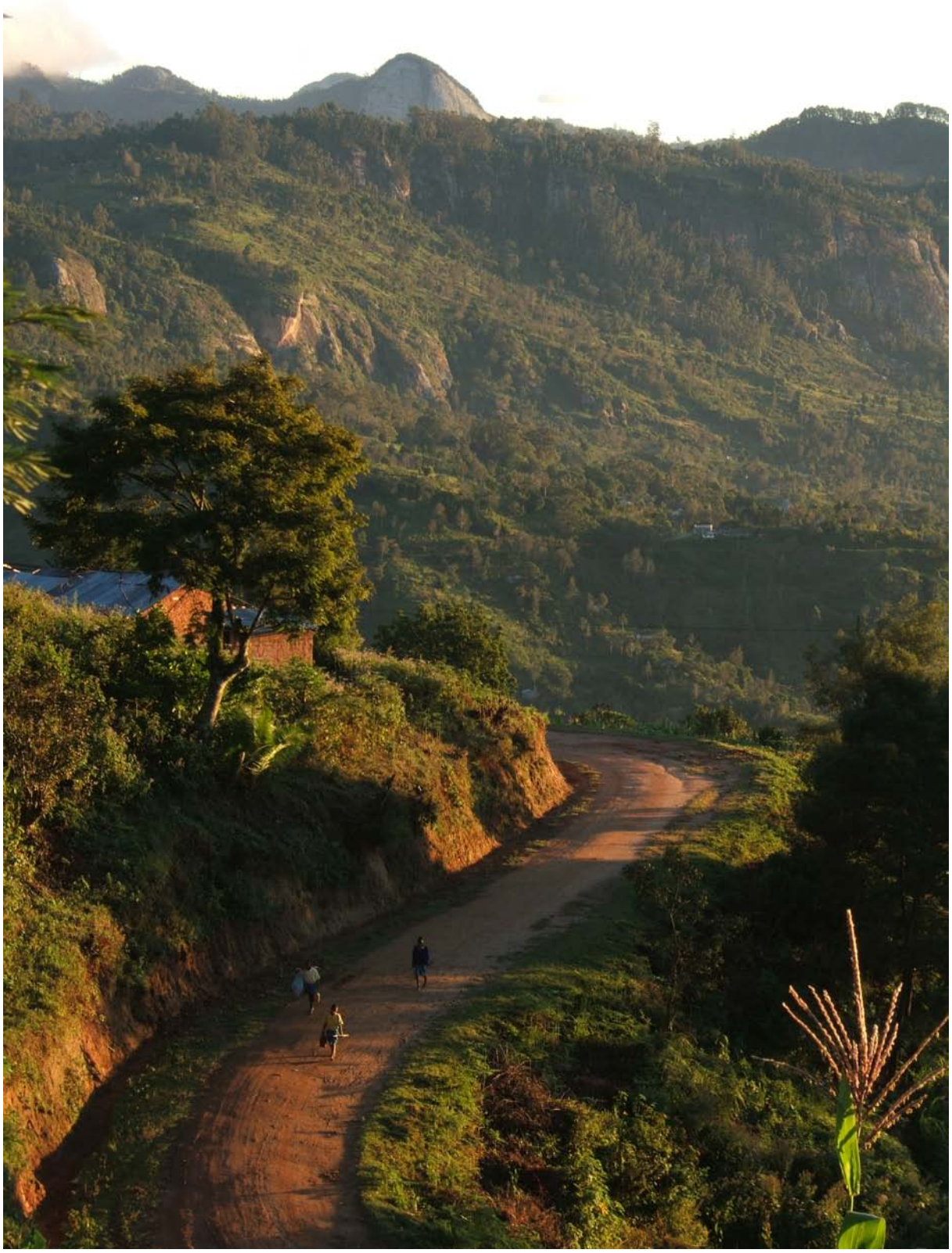

Morning light in the Taita Hills. © Tom Callens

Habitat loss and fragmentation typically result in smaller and more isolated populations of plants and animals, which are further suppressed by stochastic, demographic and environmental processes, synergistically leading to population extinctions and worldwide biodiversity loss. Currently, few in depth studies exist on genetic and demographic effects of habitat loss and fragmentation in tropical ecosystems, despite the fact that conservation implications may differ largely in tropical and temperate-zone species. Therefore, we conducted research in the isolated Taita Hills in south-east Kenya with the implicit aim of advancing our understanding on how tropical rainforest fragmentation may lead to population fragmentation in cooperatively breeding, forest-restricted bird species. To achieve this aim, we studied genetic and demographic signatures of rainforest fragmentation on populations of the cabanis's greenbul (*Phyllastrephus cabanisi*), the results of which are described in five consecutive chapters.

In Chapter 1, historical shifts in mobility in three *P. cabanisi* populations were evaluated against those of six sympatric forest bird species, by comparing species-specific levels of past population differentiation, estimated from sets of microsatellite genotypes, with contemporary dispersal rates, estimated from multi-strata capture-recapture models. In *P. cabanisi*, severe fragmentation of the original indigenous forest cover resulted in moderate historical loss of mobility over time, combined with genetic subdivision into three distinct clusters.

In Chapter 2, shifts in demographic and genetic properties were quantified within and between five *P. cabanisi* populations over a recent time interval. Contrary to our expectations, genetic and demographic spatiotemporal analyses could not substantiate a decrease in between-fragment connectivity over a more recent, fifteen years time span. Rather, contemporary populations showed higher levels of genetic variation and admixture, and effective population sizes remained largely equal or even showed a weak tendency to increase with time.

In Chapter 3, spatial and temporal variation in predation rates on natural nests of *P. cabanisi* were described in two contrasting forest fragments during three consecutive breeding seasons. Within fragments, predation levels matched the typical high predation rates on tropical bird species. However, daily predation rates on *P. cabanisi* nests in the Taita Hills were much lower than in similarly-structured forests in a more pristine part of the Eastern Arc Mountains (East Usambara). Predation rates strongly varied in space and time, and a model that combined habitat-, edge- and timing effects was best supported by our data. Nest predation rates increased from the forest edge towards the interior, supporting the notion of an inverse edge effect.

In Chapter 4, the social breeding system of *P. cabanisi* was examined by means of a detailed camera survey. Results revealed complex breeding behavior in *P. cabanisi* with a considerable amount of breeding pairs assisted by helpers, characterizing the species as a cooperative breeder. Through detailed quantification of variation in food provisioning rates at nests with and without helpers, we found that breeding females most likely adopt a load-lightening strategy. They reduce provisioning rates to nestlings when assisted by helpers, which might positively affect their fitness in terms of increased breeding attempts, condition and/or survival.

In Chapter 5, the genetic signature of forest fragmentation was revisited by adopting an individual-based approach. At local scale, we revealed fine-grained spatial patterns of positive local genetic structure, consistent with behavioral observations of natal philopatry. At a landscape scale, individual-based auto-correlation values also showed restricted dispersal, with the decreasing values over time supporting our other findings of increased gene flow over the past decade. Results of this study confirm that individual-based genetic analyses at multiple spatial scales can provide a powerful alternative to population-level analyses.

Based on genetic and demographic results of this doctoral study, we concluded that even though human-induced habitat changes in the Taita archipelago seem to have impacted *P. cabanisi* populations via moderate historical mobility loss and genetic population subdivision, shifts in the genetic population structure over the last decade at least hint towards population reconnection. Our findings are promising in the fact that the detrimental effects of forest fragmentation might be reversible, given that increased dispersal and gene flow at metapopulation level seem to dampen demographic and genetic effects of forest loss and

degradation at the local population level. This doctoral study hence supports the recommendation that conservation of fragmented populations of K-selected Afrotropical passerines requires action both within habitat fragments and at the landscape level. Within fragments, habitat loss and deterioration should be kept at a minimum in order to maintain adequate population growth and emigration to other populations. Across fragments, efforts should be made to maximize landscape connectivity in order to allow birds to move between fragments. Finally, although the demographic and genetic implications of cooperative breeding could not be evaluated thoroughly in this study, the initial detection of helpers at the nest provides a potential source for various future explorations on the evolution, consequences and conservation implications of such complex behavior.

Habitatverlies en -fragmentatie leiden meestal tot kleinere en meer geïsoleerde planten- en dierenpopulaties. Deze kleine populaties komen extra onder druk te staan ten gevolge van stochastische, demografische en omgevingsprocessen. De combinatie van deze processen is groter dan de effecten van elk afzonderlijk, wat uiteindelijk leidt tot uitsterven van populaties en biodiversiteitsverlies op wereldwijde schaal. Alhoewel tropische en gematigde soorten sterk verschillen in levensgeschiedeniskenmerken, is er in tropische ecosystemen een tekort aan gedetailleerde studies over genetische en demografische effecten van habitatverlies en -fragmentatie alsook over de mogelijke conservatie-implicaties. Daarom voerden we onderzoek uit in de geïsoleerde Taita Hills in Zuidoost-Kenia, met als hoofddoel na te gaan in welke mate tropische regenwoudfragmentatie leidt tot populatiefragmentatie in een coöperatief broedende, bosafhankelijke vogelsoort. Om die vraag te beantwoorden bestudeerden we genetische en demographische effecten van regenwoudfragmentatie in populaties van de cabanis's greenbul (*Phyllastrephus cabanisi*), waarvan de resultaten in vijf opeenvolgende hoofdstukken uiteengezet zijn.

In hoofdstuk 1 werden historische veranderingen in mobiliteit in drie *P. cabanisi* populaties geëvalueerd ten opzichte van zes soortgelijke bosvogelsoorten. Er werd een vergelijking gemaakt van soortspecifieke niveaus van historische populatiedifferentiatie, geschat op basis van microsateliet-genotypes, met huidige dispersie-snelheden, geschat op basis van multi-strata vangst-hervangst modellen. Hieruit bleek dat *P. cabanisi* door sterke fragmentatie van de originele endemische bossen, een matig mobiliteitsverlies doorheen de tijd heeft gekend, gecombineerd met genetische opsplitsing in drie aparte groepen.

In hoofdstuk 2 werden verschuivingen in demografische en genetische eigenschappen gekwantificeerd binnen en tussen vijf *P. cabanisi* populaties over een recent tijdsinterval. In tegenstelling tot onze verwachtingen vertoonden genetische en demografische spatio-temporele analyses over een meer recente tijdspanne van 15 jaar geen daling in connectiviteit tussen fragmenten. Integendeel, de huidige populaties bezitten een hogere mate van

genetische variatie en zijn sterker met elkaar vermengd. Ook de effectieve populatiegroottes bleven grotendeels gelijk of vertoonden een verhoogde trend.

In hoofdstuk 3 werden ruimtelijke en tijdelijke variatie in predatie op natuurlijke nesten van *P. cabanisi* gedurende drie opeenvolgende broedseizoenen in twee contrasterende bosfragmenten beschreven. In de fragmenten stemde predatie overeen met de typische hoge predatie niveaus bij tropische vogelsoorten. De dagelijkse predatie op *P. cabanisi* nesten in de Taita Hills was echter veel lager dan in vergelijkbare bossen in een meer ongerept deel van de Eastern Arc Mountains (East Usambara). Het model bestaande uit een combinatie van habitat-, rand- en tijdseffecten verklaarde onze gegevens het best, met sterk variërende predatie in ruimte en tijd. Nestpredatie nam toe van de bosrand naar het midden van het bos, wat het idee van een 'omgekeerd rand-effect' ondersteunt.

In hoofdstuk 4 werd het sociale broedsysteem van *P. cabanisi* onderzocht door middel van een gedetailleerd videocamera onderzoek. De resultaten onthulden complex broedgedrag in *P. cabanisi* met een aanzienlijke hoeveelheid broedparen bijgestaan door helpers, wat de soort duidelijk karakteriseert als een coöperatieve broeder. Via een gedetailleerde kwantificering van de variatie in voedselaanvoer tussen nesten met en zonder helpers stelden we een taakverlichtende strategie vast bij de broedende wijfjes. Ze verlaagden hun persoonlijk aandeel in de voedselaanvoer wanneer ze werden bijgestaan door helpers, wat op lange termijn een positieve invloed zou kunnen hebben op hun fitness in verband met een verhoogd aantal broedpogingen, betere conditie en / of hogere overlevingskansen.

In hoofdstuk 5 werden de genetische effecten van bosfragmentatie opnieuw onderzocht via een individueel-gerichte aanpak. Op lokaal niveau toonden we de aanwezigheid aan van een fijnkorrelig ruimtelijk patroon van plaatstelijke positieve genetische structuur, wat overeenstemde met observaties van eerstejaarsvogels die in hun geboortehabitat bleven. Ook op landschapsschaal gaven individueel-gebaseerde auto-correlatiewaarden een beperkte spreiding van genen aan. Deze waarden namen echter af doorheen de tijd, wat onze eerdere bevindingen ondersteunde inzake toegenomen genetische uitwisseling over het afgelopen decennium. De resultaten van deze studie bevestigen dat individueel-gebaseerde genetische analyses op verschillende ruimtelijke schalen een krachtig alternatief kunnen zijn voor analyses op populatieniveau.

Op basis van genetische en demografische resultaten van dit doctoraatsonderzoek konden we besluiten dat desondanks het feit dat menselijk geïnduceerde habitatveranderingen in de Taita archipel hebben geleid tot middelmatig historisch mobiliteitsverlies en genetische populatie-opsplitsing in *P. cabanisi*, er toch aanwijzingen zijn in verschuivingen van de genetische populatiestructuur gedurende het laatste decennium, die populatie-herconnectie laten vermoeden. Onze bevindingen zijn veelbelovend daar de nadelige effecten van bosfragmentatie mogelijks omkeerbaar zijn, gezien de verhoogde dispersie en genetische uitwisseling op metapopulatieniveau, demografische en genetische effecten van bosverlies -en degradatie op populatieniveau lijken te temperen. Dit doctoraatsonderzoek ondersteunt de aanbevelingen dat voor de bescherming van K-geselecteerde Afrotropische vogelsoorten actie vereist is zowel op lokaal niveau binnen habitatfragmenten als op landschapsniveau. Binnen fragmenten zou habitatverlies en -degradatie tot een minimum moeten beperkt worden om populatie-aangroei en emigratie naar andere fragmenten te garanderen. Tussen fragmenten zouden inspanningen moeten gedaan worden om de landschapsconnectiviteit te optimaliseren zodat de vogels zich voldoende tussen de fragmenten kunnen verplaatsen. Tot slot is de initiële ontdekking van coöperatief broedgedrag (helpers aan het nest) bij deze vogelsoort een potentiële bron voor veelzijdige toekomstige studies inzake de evolutie, gevolgen en conservatie-implicaties van dit complexe gedrag.

Sunset behind Mount Kilimanjaro, seen from Chawia forest in the Taita Hills. © Tom Callens


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